

Pub # P2-388

Session Information POSTER SESSION: CLINICAL - Hypopituitarism (1:30 PM-3:30 PM)

Title Factors Associated with Obesity in Children with Septo-Optic Dysplasia

Author String RRD Coletta, C Redington, E Webb, MT Dattani
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Body

Introduction: Septo-optic dysplasia (SOD) is a rare congenital heterogeneous disorder with a prevalence ranging from 6.3 to 10.9 per 100,000. SOD is defined by the presence of any two of the following features; midline forebrain defects, optic nerve hypoplasia and hypopituitarism. SOD is equally prevalent in both genders. Obesity is a significant problem in SOD; however risk factors for obesity have not previously been explored.

Aim: To establish the factors associated with obesity in SOD.

Patients and methods: Retrospective case note review of 113 children with SOD attending clinics at Great Ormond Street Hospital. Children were divided into two groups based on their body mass index. Thirty infants/children (25.5%) had a BMI > 2 standard deviations (SDS) and 83 infants/children (73.5%) a BMI < 2 SDS from the normal. Data including hormonal insufficiencies (growth hormone, TSH, ACTH deficiency and diabetes insipidus), magnetic resonance imaging (MRI) brain appearances [small anterior pituitary (SAP), ectopic or absent posterior pituitary (EPP/APP), bilateral optic nerve hypoplasia (ONH), abnormal septum pellucidum (SP) and corpus callosum (CC)], level of visual impairment (none, mild, moderate, severe, blind), presence or absence of autism and/or a sleep disorder were recorded in all patients. Statistical analysis was performed in SPSS version 14 and independent samples T-test performed.

Results: 113 children with SOD (65 male) were identified. Children with a BMI > 2 SDS above the normal had a higher frequency of hormonal deficiencies, sleep disruption, ectopic or absent PP and bilateral ONH ($p < 0.05$). Small anterior pituitary, degree of visual impairment and the presence of autism were not associated with obesity in children with SOD.

Conclusion: Identification of the factors associated with obesity in SOD, [hormonal deficiencies, sleep abnormalities, abnormal posterior pituitary (EPP/APP) and bilateral ONH] may allow us to better develop prospective targeted intervention strategies in this group of patients.

Nothing to Disclose: RRDC, CR, EW, MTD

Pub # P2-389

Session Information POSTER SESSION: CLINICAL - Hypopituitarism (1:30 PM-3:30 PM)

Title Ectopic Posterior Pituitary Associated with Growth Hormone and Gonadotropin Deficiency

Author String F Pink, J Kumar, P Goulden
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Body This patient first presented aged 6 years with parental concerns over his growth which had been slowing since the age of 3. When first seen height had dropped below the 0.4th centile, well below the mid parental height 75th centile. Clonidine growth hormone stimulation test demonstrated maximal growth hormone of only 6mu/L confirming growth hormone deficiency. A pituitary/hypothalamic MRI scan showed a likely ectopic posterior pituitary.

Investigation of the rest of pituitary function suggested an isolated growth hormone deficiency. The patient was treated with synthetic growth hormone for eleven years, until it was stopped at the age of 17 having achieved a height on the 50th centile.

However the patient also experienced delay in puberty (arrested at Tanner stage III). A diagnosis of ongoing growth hormone deficiency and hypogonadotropic hypogonadism was made on the basis of repeat pituitary function tests (Testosterone 1.8, LH 2.1, FSH <2, IGF-1 9.4, prolactin 198, TSH 3.4, free T3 4.8, free T4 13.2). The patient was recommenced on synthetic growth hormone to augment pubertal induction monthly intramuscular synthetic testosterone injections. The finding of an ectopic posterior pituitary may be associated with multiple hormone deficiencies and these patients need ongoing, lifelong evaluation.

Nothing to Disclose: FP, JK, PG

Pub # P2-390

Session Information POSTER SESSION: CLINICAL - Hypopituitarism (1:30 PM-3:30 PM)

Title An Ectopic Posterior Pituitary Case with Multiple Hormone Deficiencies

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Body 19 year old female patient was admitted to our endocrinology clinic with symptoms of fatigue, retardation of growth and development, and amenorrhea. Basal hormonal data and hormone-stimulating tests revealed impaired secretion of growth hormone (GH) and gonadotropins. There was also deterioration in thyroid axis. ACTH and cortisol response was in normal ranges during insulin tolerance test. Urine output and density was normal, she did not have symptoms of polyuria. With these data, to see if there is any pituitary pathology, magnetic resonance imaging (MRI) was obtained. There was anterior pituitary hypoplasia, pituitary stalk could not be identified, and there was nodular, homogenous lesion at the hypothalamus level which was compatible with ectopic posterior pituitary (EPP) at MRI with gadolinium injection. EPP is a complex disease its natural course and etiology is not well known. Patients should be investigated with precise imaging techniques and laboratory tests. The patients with absence of pituitary stalk visibility are afflicted with a more severe form of adenohypophysis hypoplasia and with multiple pituitary hormone deficiencies. Also the patients must be reassessed after the first evaluation, because progression to complete pituitary hormone deficiency may occur progressively. The patients with visible pituitary stalk seem to have a better endocrinological prognosis. The case is presented because the clinical and follow-up data about this syndrome is limited.

Nothing to Disclose: GO, AG, DD, MB, MA, BDM

Pub # P2-391

Session Information POSTER SESSION: CLINICAL - Hypopituitarism (1:30 PM-3:30 PM)

Title Pituitary Gland Abnormalities as a Complication of Wegener Granulomatosis

Author String AA Ekhzaimy
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Body
Background: Wegener's granulomatosis (WG) is a small vessel vasculitis characterized by ANCA-mediated vasculitis (antineutrophil cytoplasmic antibody), necrosis and granuloma. Although it could affect the central nervous system, very rare complications include diabetes insipidus and pituitary abnormalities. We describe findings in one case of pituitary involvement in Wegener's granulomatosis that corresponds with observations reported in the literature.
Clinical case: A 38-year woman presented with epistaxis, arthralgia and hemoptysis in 2007. Initial test results were consistent with Wegener's granulomatosis: 24-hr urine protein was high (0.3027 g/day N < 0.15 g/day), ESR was elevated (120 mm/hr, N < 17 mm/hr), ANA was positive (1:40) and cANCA was positive (1:80). A chest X-ray and a chest CT scan revealed left upper lobe lung cavitation. A nasal biopsy confirmed the diagnosis of Wegener's granulomatosis (presence of necrotizing granuloma) followed by a renal biopsy supporting similar findings. She was treated with pulse cyclophosphamide and prednisolone for six months and maintained on prednisolone 5 mg/day and azathioprine 125 mg/day. The patient developed polyuria and polydipsia in 2008. She could not complete the water deprivation test because of significant polyuria. Biochemical testing showed high serum sodium of 149 mmol/L (135-145 mmol/L), urine osmolality was low 69 mosm/Kg (N: 50-1200 mosm/Kg), serum osmolality was 287.97 (N: 278-305 mosm/Kg), 24-hour intake was 5 L and urine output was 8 L/day, with no evidence of diabetes mellitus. TSH 3.11 (0.25-5 mIU/L), FT4 14.65 (10.3- 25.8 pmol/L), prolactin 678.3 (102- 496 mIU/L), and LH/FSH were normal. An MRI showed a focal central lesion that was hypointense on T1 and hyperintense on T2 with an absent posterior pituitary bright spot on T1 suggesting the pituitary involvement in WG. The woman's symptoms declined markedly after we started her on desmopressin nasal spray 10 mcg twice daily. A follow-up MRI after one year demonstrated a normal pituitary appearance with persistent absence of a posterior bright spot.
Conclusion: Diabetes insipidus and radiological changes in the pituitary resulting from WG are rare but well-described complications, in a few reported cases. Clinicians should consider this possibility when patients present with polyuria and polydipsia. Pituitary hormonal and radiological screening is essential if patients present with anterior pituitary hormonal dysfunction symptoms.

Nothing to Disclose: AAE

Pub # P2-392

Session Information POSTER SESSION: CLINICAL - Hypopituitarism (1:30 PM-3:30 PM)

Title Identification of Candidate Autoantigens in Lymphocytic Hypophysitis: Immunoscreening of a Pituitary cDNA Library and Development of Immunoprecipitation Assays

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Body

Background: Lymphocytic hypophysitis is an organ-specific autoimmune disease characterised by infiltration of lymphocytes into the pituitary gland. A specific and sensitive serological test currently does not exist to aid in the diagnosis of this disease.

Objective: To identify target autoantigens in lymphocytic hypophysitis and to develop a diagnostic assay to determine the frequency and specificity of immunoreactivity to these proteins.

Design/Methods: A pituitary cDNA expression library was immunoscreened using serum from four previously characterized patients with lymphocytic hypophysitis. Promising cDNA clones from the screening along with previously identified autoantigens pituitary gland specific factor 1a and 2 (PGSF1a, PGSF2) and neuronspecific enolase (NSE) were tested in an ITT (*in vitro* transcription translation) immunoprecipitation assay. The corticotroph-specific transcription factor, T-box 19 was also investigated as a candidate autoantigen.

Results: Significantly positive autoantibody reactivity against T-box 19 was found in 9 of 86 (10.5%) lymphocytic hypophysitis patients versus 1 of 90 (1.11%) controls ($p=0.019$, [chi]² Yates correction). The reactivity against T-box 19 was not specific for lymphocytic hypophysitis since antibodies were also found in sera from patients with other autoimmune endocrine diseases. Autoantibodies against chromodomain helicase DNA binding protein 8 (CHD8), presynaptic cytomatrix protein (piccolo), Ca²⁺-dependent secretion activator (CADPS), PGSF2 and NSE were also detected in sera from lymphocytic hypophysitis patients however not more frequently than among controls.

Conclusions: T-box 19, a corticotroph-specific transcription factor, was identified as a minor target autoantigen in 10.5% of lymphocytic hypophysitis patients. Immunoscreening of a pituitary cDNA expression library was found to be a powerful method in identifying multiple potential target autoantigens.

Nothing to Disclose: CJAS, SB, CB, PR, RS, JD, AAK-Z, OK, PC

Pub # P2-393

Session Information POSTER SESSION: CLINICAL - Hypopituitarism (1:30 PM-3:30 PM)

Title Histiocytosis Causing Diabetes Insipidus

Author String CDP Blankenburg, EDS Portes, RA Guerra, ER Barbosa, LB de Sa, AR Andrade, DL Belino, VC Machado
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Body Diabetes insipidus (DI) is an uncommon disorder characterized by polyuria (diuresis greater than 40ml/kg/day in adults and 100 ml/kg/day in children), usually accompanied by polydipsia. There are four mechanisms that cause the disease: failure to synthesize and/or secrete antidiuretic hormone - ADH (central diabetes insipidus) inadequate renal response to ADH (nephrogenic diabetes insipidus), excessive water intake (primary polydipsia), accelerated metabolism of ADH during pregnancy (transient diabetes insipidus in pregnancy). The central diabetes insipidus has many causes which may be primary - genetic abnormalities, secondary - trauma, neoplasms, surgery, infections, vascular lesions, Langerhans cell histiocytosis, sarcoidosis, or idiopathic causes. We report a case of a 34-year-old man, 85kg, smoker, with a history of polyuria (diuresis 130ml/kg/dia), polydipsia, and plasma and urine osmolality: 282mOsm/kg and 60 mOsm/kg, respectively. A water restriction test was stopped after 4 hours due hypernatremia (sodium = 150mEq/L). He had a significant increase in urine osmolality (from 67mOsm/kg to 273) and normalization of sodium levels (sodium = 146) after application of intranasal desmopressin, confirming central DI. Later, he was diagnosed with Pulmonary Langerhans cell histiocytosis (PLCH) based on clinical history and chest tomography. The PLCH, a rare disease of unknown etiology, usually occurs in young adults, smokers, with predominant pulmonary involvement. The main symptoms are dry cough and dyspnea. In relation to extrapulmonary involvement, the bone and skin lesions, and the involvement of the hypothalamic-pituitary region with diabetes insipidus are the disturbances more often seen in adults. The diagnosis can be established in patients with compatible clinical findings that show typical characteristics of the disease on chest tomography. Until the present moment, no therapeutic proved efficient in the treatment of PLCH and the beneficial effect of smoking cessation in the evolution of disease has been demonstrated only in some reports. Therefore, we sought to emphasize the importance of suspicion of these uncommon diseases to diagnose them correctly.

Nothing to Disclose: CdPB, EdSP, RAG, ERB, LBdS, ARA, DLB, VCM

Pub # P2-394

Session Information POSTER SESSION: CLINICAL - Hypopituitarism (1:30 PM-3:30 PM)

Title Central Hypothyroidism Presenting after Predeployment Anthrax and Smallpox Vaccination

Author String K Kinnaird, RA Vigersky, J Williams
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Body **Background:** We evaluated a patient who developed central hypothyroidism after predeployment anthrax and smallpox vaccinations. The vaccinations may have triggered this predisposed patient to cross an immunological threshold, thereby extending autoimmunity to the pituitary.

Clinical case: A 23 year-old Active-Duty woman presented with polyarthralgias and myalgias several weeks after receiving anthrax and smallpox vaccinations in October 2009. Other symptoms were decreased appetite, alternating heat and cold intolerance, fatigue, insomnia, constipation, xerosis, and brittle nails. She had no significant past medical history. Her sister and mother had hypothyroidism. Rheumatology evaluation, to include creatinine kinase and aldolase, was normal except for ANA titer 1:160. She was referred to us in January 2010 for positive TPO 49 (normal <35 IU/mL) and thyroglobulin (TG) 398 (normal <20 IU/mL) antibodies and a normal TSH 2.99 (0.27-4.2 mIU/mL). Physical examination was normal. FT4 by analog immunoassay and direct dialysis was low (0.57 ng/dl - normal 0.77-1.61 ng/dL and 0.7 ng/dl - normal 0.8-2.7 ng/dL, respectively), but her TSH was normal (2.07 mIU/ml). We interpreted these data as central hypothyroidism rather than primary hypothyroidism from Hashimoto's thyroiditis. Hormonal evaluation of the remaining pituitary axis and MRI of the sella/pituitary were normal. Her 2007 Department of Defense serum repository blood sample (frozen at -30[deg]C) had negative TPO antibodies, positive TG antibodies 248 IU/mL and normal FT4 by direct dialysis 2 ng/dL; the sample was insufficient for measuring TSH. Her symptoms dramatically improved with l-thyroxine 88 mcg daily and normalization of FT4 (0.92 ng/dL by analog immunoassay and 1.9 ng/dL by direct dialysis).

Conclusion: Onset of central hypothyroidism after smallpox and anthrax vaccinations has not been previously reported. The temporal association in our patient does not prove causality, but the development of TPO antibodies after vaccination in 2009 suggests vaccination may have induced further autoimmunity affecting both the pituitary and thyroid. Since similar observations have been made with regard to the development of Type 1 diabetes, further investigation into potential mechanisms is warranted.

The views expressed are those of the authors and do not necessarily reflect the official policy or position of the Department of the Army, Navy, Air Force, Marines, Department of Defense, nor the US Government.

Nothing to Disclose: KK, RAV, JW

Pub # P2-395

Session Information POSTER SESSION: CLINICAL - Hypopituitarism (1:30 PM-3:30 PM)

Title Burkitt Lymphoma Involving the Pituitary Gland: Clinical Presentation and Management

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Body

Introduction
Painful ophthalmoplegia and hypopituitarism can result from any process causing mass effect involving the cavernous sinus, hypothalamus or pituitary gland, but Burkitt's lymphoma is a very rare culprit. Corticosteroids are often first line treatment for these patients however, glucocorticoid-induced apoptosis in lymphocytes can reduce the diagnostic yield at time of biopsy.

Clinical Case
A 19-year old woman presented with gradual diplopia, right eye ptosis, periorbital swelling, and severe fatigue. On examination she had proptosis, extensive periorbital edema, ptosis in the right eye, postural hypotension, and a palpable left breast mass. Brain MRI, MRA and MRV revealed a lobulated enhancing 2.9 x 2.5 cm parasellar mass extending into the suprasellar region and involving the right cavernous sinus. Biochemical evaluation demonstrated hypopituitarism: cortisol 0.6 [μ]g/dl, ACTH 9 pg/ml, TSH 0.10 uIU/ml, Free T4 0.7 ng/dl, FSH 0.5 mIU/ml, LH <0.1 mIU/ml, IGF-1 164 ng/ml, PRL 38.3 ng/ml. Lymphoma involving the skull base, sella and cavernous sinus can present with rapid onset panhypopituitarism and cavernous sinus syndrome. Steroids were initially withheld until biopsy of the mass could be completed, but she rapidly developed right eye pain and worsening lethargy requiring prompt treatment with dexamethasone. An endonasal transsphenoidal biopsy was performed, but areas of focal necrosis were present. Ultrasound-guided core biopsy and fine needle aspirate of the left breast lesion showed classic morphologic and immunophenotypic features of Burkitt lymphoma. The characteristic rearrangements of *c-myc* were detected by FISH. CSF and iliac crest marrow were not involved. Treatment was initiated with intrathecal conventional CODOX-M-IVAC chemotherapy, levothyroxine and dexamethasone. Complications included central diabetes insipidus, requiring DDAVP. Post treatment imaging showed complete resolution of pituitary mass and no FDG-avid disease on PET/CT scan. She regained complete right eye function. Adrenal insufficiency resolved. She continued thyroid hormone replacement, DDAVP and has remained amenorrheic.

Conclusion
Burkitt's lymphoma involving the pituitary gland is a rare clinical entity, which in this case presented with painful ophthalmoplegia and hypopituitarism. Our case illustrates the rapid clinical presentation of a malignant sellar mass and exemplifies the difficulty of pathologic interpretation after glucocorticoid administration.

Nothing to Disclose: IODC, JF, RB, GEV, LMC

Pub # P2-396

Session Information POSTER SESSION: CLINICAL - Hypopituitarism (1:30 PM-3:30 PM)

Title Pituitary Function after Severe Traumatic Brain Injury (TBI) in Northern Sweden

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Body Background: Several recent studies indicate that hypopituitarism following traumatic brain injury (TBI) may be much more common than previously thought, with a prevalence of 15-68% (1). Since symptoms of hypopituitarism are often diffuse and difficult to separate from TBI-related asteno-emotional syndrome recommendations of hormone assessment after TBI has been proposed (2).
At Umea University Hospital severe TBI is treated according to an ICP-targeted protocol based on aggressive neurosurgery and the Lund concept (3), resulting in a mortality of less than 15%.
The aim of the current study was to investigate pituitary function in a well-defined cohort of patients with severe TBI treated according to the Lund concept and attempt to identify predictive factors for developing pituitary hormone insufficiency and its relation to long-term functional outcome.
Patients and methods: Thirty-two patients, 15-70 years, with severe TBI (Glasgow Coma Scale, GCS<9) were assessed for pituitary function >12 months after TBI. Pituitary assessment included protocol based clinical examination by an endocrinologist, fasting blood samples for TSH, free T4, FSH, LH, testosterone+SHBG (men) or estradiol (women), IGF-1 and prolactin. The HPA-axis was assessed by an ACTH-test, GH-axis by GHRH-Arginine-test (n=29) and ADH by urinary- and serum-osmolality after over-night thirst. Strict definitions of pituitary insufficiency were used and only abnormal results verified by repeated testing were accepted.
Results: At long-term pituitary assessment (18 months-7 years after TBI) no patient had a post-traumatic central hypothyroidism, adrenocortical insufficiency or diabetes insipidus. 3/20 men (15%) had a hypogonadotropic hypogonadism. In the 7 assessable women (not taking exogenous estrogens) all had a normal gonad axis. GH-deficiency was found in 2/29 (7%) of the patients. No significant associations could be established between clinical or physiological parameters in the acute phase of TBI and chronic pituitary insufficiency. In the male patients there was a positive correlation between testosterone levels at follow-up and functional outcome assessed by extended Glasgow Outcome Scale (GOS-E) at 3, 12 and 24 months after TBI.
Conclusions: Pituitary insufficiency occur after severe TBI treated according to the Lund concept, however at a lower frequency than reported in most studies. Testosterone deficiency is the most common and levels appear to be associated to functional outcome.

(1) Schneider HJ et al., JAMA 2007; 298:1429
(2) Ghigo E et al., Brain Inj; 2005; 19:711
(3) Grande PO, Intensive Care Med 2006; 32:1475

Sources of Research Support: Investigator-Initiated Research Grant by Pfizer, Inc.

Disclosures: PD: Principal Investigator, Pfizer, Inc. CM: Researcher, Pfizer, Inc. ZO: Researcher, Pfizer, Inc. L-ODK: Researcher, Pfizer, Inc.

Pub # P2-397

Session Information POSTER SESSION: CLINICAL - Hypopituitarism (1:30 PM-3:30 PM)

Title Pituitary Dysfunction in Adult Patients after Cranial Radiotherapy: A Systematic Review and Meta-Analysis

Author String NM Appelman-Dijkstra, NE Kokshoorn, OM Dekkers, NR Biermasz, KJ Neelis, JA Romijn, JWA Smit, AM Pereira
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Body
Background: Cranial radiotherapy for non-pituitary tumors is an important cause of hypopituitarism. However, the prevalence of reported hypopituitarism varies considerably between studies and structured endocrine assessments are currently not incorporated in long term cancer survivor follow-up programs.
Methods: Systematic review and meta-analysis of studies reporting prevalence of hypopituitarism in adults after cranial radiotherapy for non-pituitary tumors. PubMed, EMBASE, Web of Science, Cochrane and references of key articles were searched to identify potentially relevant studies. A meta-analysis was performed with an exact likelihood approach using a logistic regression with a random effect at the study level.
Results: Eighteen studies were included with a total of 873 patients. These included 608 patients treated for nasopharyngeal cancer (70%) and 265 for intracerebral tumors. The total radiation dose ranged from 14-83 Gy and 40-97 Gy for nasopharyngeal and intracerebral tumors, respectively. The point prevalence of any degree of hypopituitarism was 0.66 (95%CI 0.55-0.76). The prevalence of deficiency of growth hormone was 0.45 (95%CI 0.33-0.57), of LH and FSH 0.3(95%CI 0.23-0.37), of TSH 0.25(95%CI 0.16-0.37), and of ACTH 0.22 (95%CI 0.15-0.3), respectively. The prevalence of hyperprolactinemia was 0.33(95%CI 0.16-0.56). There were no differences between the effects of radiotherapy for nasopharyngeal versus for intracerebral tumors.
Conclusion: Hypopituitarism is highly prevalent in adult patients after cranial radiotherapy for nasopharyngeal tumors and cerebral tumors. Therefore, all patients treated by cranial radiotherapy should have a lifelong structured periodical assessment of pituitary functions.

Nothing to Disclose: NMA-D, NEK, OMD, NRB, KJN, JAR, JWAS, AMP

Pub # P2-398

Session Information POSTER SESSION: CLINICAL - Hypopituitarism (1:30 PM-3:30 PM)

Title Cranial Radiotherapy Is Associated with a Higher Visceral to Subcutaneous Fat Ratio in Men Treated for Pituitary Insufficiency

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Body

Background
Previous studies have shown that hypothalamic neurons are involved in the control of body fat distribution. As hypothalamic nuclei are radiosensitive, we hypothesized that a history of cranial radiotherapy (CRT) in patients with pituitary insufficiency is associated with changes in body fat distribution independent of hormonal status.

Methods
Consecutive male subjects visiting our Endocrine Outpatient Clinic for pituitary insufficiency, who had previously been treated for a sellar tumor, were invited to participate. A standardized single slice abdominal CT-scan at the level of the fourth lumbar vertebra was performed to determine the visceral fat area, subcutaneous fat area and visceral to subcutaneous fat ratio. In addition, we measured total fat percentage measured with bioelectrical impedance analysis, body mass index, serum testosterone, IGF-1 and cortisol concentrations.

Results
We included 16 men with previous CRT and 19 men without (age 62 (35 - 79) vs 59 (24 - 79) y, $p = 0.161$; body mass index 28.5 (21.8 - 37.1) vs 29.9 (24.2 - 41.9) kg/m²; $p = 0.317$). Subjects with CRT had a smaller subcutaneous fat area (225.1 (71.1 - 480.7) vs 269.0 (133.2 - 59.9) cm², $p = 0.022$) and a higher visceral to subcutaneous fat ratio (0.79 (0.39 - 1.55) vs 0.63 (0.23 - 0.88), $p = 0.001$) than subjects without CRT. There were no differences in visceral fat area, total fat percentage, serum testosterone, serum IGF-1 and serum cortisol 240 min following hydrocortisone ingestion (p -values respectively 0.271, 0.193, 0.659, 0.403 and 0.595).

Conclusion
In men treated for pituitary insufficiency, previous cranial radiotherapy is associated with a higher visceral to subcutaneous fat ratio. This association is independent of serum hormone levels.

Nothing to Disclose: AJB, AA, HWV, EF, PHB

Pub # P2-399

Session Information POSTER SESSION: CLINICAL - Hypopituitarism (1:30 PM-3:30 PM)

Title Risk Factors for Hypopituitarism Following Subarachnoid Hemorrhage

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Body

Background: Hypopituitarism is a potential consequence of subarachnoid hemorrhage (SAH). The aim of this study was to investigate the prevalence of endocrine abnormalities in patients with SAH and determine which clinical parameters may be associated with hypopituitarism.

Methods: Pituitary function was evaluated in 25 patients (18 females, 7 males), aged 51 ± 11 years, within 3-24 months of SAH. Hormonal assessment included prolactin, thyroid and gonadal testing, and a low dose (1 mcg) cortrosyn stimulation test (normal > 15 mcg/dl at 30-60 minutes). Growth hormone deficiency (GHD) was assessed with GHRH-Arginine or glucagon stimulation tests (moderate to severe GHD defined by peak GH < 10 ng/ml and severe GHD defined by peak GH < 3 ng/ml).

Results: Hypopituitarism (at least one axis) was present in 15 (60 %) subjects, including 9 (36%) with moderate to severe GHD, 4 (16%) with severe GHD, 5 (20%) with adrenal insufficiency, 3 (12%) with hypothyroidism, and 1 (4%) with hypogonadism. Eight (32%) patients were deficient in two axes, while no participants had deficiencies in three axes. Fisher grade 1, 2, 3, and 4 SAHs were present in 1 (4%), 3 (8%), 18 (72%), and 3 (8%) subjects, respectively. Thirteen (52%) individuals had post-SAH vasospasm, and 10 (40%) patients underwent intervention including lamina terminalis fenestration (LTF). Fisher grade ($r = -0.45$, $p = 0.014$) and LTF ($r = 0.48$, $p = 0.011$) correlated with hypopituitarism in SAH patients, while the Hunt-Hess score, EVD placement, aneurysm location, and vasospasm did not. Hunt-Hess score ($r = -0.58$, $p = 0.003$), LTF ($r = 0.42$, $p = 0.026$), and vasospasm ($r = 0.50$, $p = 0.011$) correlated with severe GHD, while only Hunt-Hess score ($r = -0.59$, $p = 0.002$) and LTF ($r = 0.62$, $p = 0.001$) correlated with moderate to severe GHD.

Conclusions: In our series, hypopituitarism was present in 60% of individuals with a history of SAH, and moderate to severe GHD was the most common anterior pituitary dysfunction. Fisher grade and a surgical procedure including LTF correlated with subsequent hypopituitarism, while the Hunt-Hess score, LTF, and vasospasm were associated with the development of severe GHD within 3-24 months of the injury. Our study indicates that patients with a history of SAH, particularly those with more severe SAH or associated vasospasm, may be at greater risk for post event hypopituitarism, especially GHD.

Disclosures: TG: Coinvestigator, Genentech, Inc. LK: Researcher, Novartis Pharmaceuticals; Speaker, Ipsen. Nothing to Disclose: RD, AH, GS

Pub # P2-400

Session Information POSTER SESSION: CLINICAL - Hypopituitarism (1:30 PM-3:30 PM)

Title Hypothalamo-Pituitary Dysfunction in Patients with Chronic Subdural Hematoma

Author String M Kosak, V Masopust, D Netuka, Z Lacinova, M Krsek, J Marek, V Hana
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Body Certain degree of hypopituitarism has been revealed in a significant number of patients who suffered traumatic brain injuries or subarachnoid hemorrhage. Microhemorrhages, necroses, tissue infarcts and vasoconstriction and probably autoimmune process are reported as mechanisms of hypothalamo-pituitary dysfunction. Assessment of hypothalamo - pituitary endocrine functions in patients with chronic subdural hematomas has not been published yet, although dysfunction of hypothalamo- pituitary unit can be expected (head trauma, compression and oedema of the brain with shifting of the midline structures).
Aims: Prospective evaluation of the hypothalamo-pituitary functions in patients with chronic subdural hematoma (SDH) immediately after surgical treatment and during one year follow-up.
Patients and methods: We have examined 48 patients (41 men, 7 women, 36-88 years old, mean 66.4 +/- SE 12.9 years) so far. Patients in the first days after evacuation of the hematoma and then 3 and 12 months after the operation are being tested. Basal levels of pituitary hormones and hormones of dependent peripheral glands, plasma cortisol during 250 mcg tetracosactide (Synacthen) test, GH after GHRH+arginin stimulation and TSH after TRH stimulation are assessed.
Results: Central hypogonadism was diagnosed in 14 male patients (34%) in the first examination. In eight of them gonadotrophic axis normalised within 3 months after the operation (four patients haven[acute]t been retested yet). Central hypogonadism was newly diagnosed three months after the operation in two patients. No hypocorticalism was present in the first examination. Borderline hypocorticalism was present after three months in one patient (maximal cortisol response in ACTH test 475 nmol/l), but the control test was normal. Severe growth hormone deficiency (GHD) was diagnosed in the first examination in 20 patients. In five of them somatotrophic axis normalised within three months after the operation, but in other four was GHD diagnosed after 3 months (9 patients haven[acute]t been retested yet). No hypothyroidism was diagnosed as a sequelae of SDH. Relation between extent of subdural hematoma and hypothalamo - pituitary dysfunction has been analysed.
Conclusions: Preliminary data from our group of patients show that chronic subdural hematoma may be accompanied with a partial pituitary dysfunction and that it can develop even with a time delay.

Sources of Research Support: Grant of Czech Ministry of Health NS 9794 - 4.

Nothing to Disclose: MK, VM, DN, ZL, MK, JM, VH

Pub # P2-401

Session Information POSTER SESSION: CLINICAL - Hypopituitarism (1:30 PM-3:30 PM)

Title Pituitary Function in Patients with Normal Pressure Hydrocephalus before and after Shunt Placement

Author String T Moin, M Bergsneider, AP Heaney
David Geffen School of Medicine at UCLA, Los Angeles, CA

Body

Introduction:
Hydrocephalus is a rare cause of endocrine dysfunction. Reports have included primary and secondary amenorrhea, precocious puberty, secondary hypothyroidism and abnormalities of growth hormone secretion. The mechanism remains unclear although dilatation of the 3rd ventricle, abnormal CSF pressures, and/or direct pressure on the pituitary stalk have been postulated. Limited data is available on pituitary function in patients with Normal Pressure Hydrocephalus (NPH). A prior small series reported endocrine dysfunction in 4 of 6 NPH patients (83.3%) and advocated that all NPH patients should undergo endocrinological evaluation before surgical intervention. The goal of this study was to evaluate pituitary function in a large NPH clinical service.

Materials and Methods:
Patients with a diagnosis of NPH referred for neurosurgical shunt placement between February 2009 and January 2010 were eligible for recruitment. The diagnosis of NPH was made according to standard criteria on the basis of history, clinical findings, imaging and in some cases, an elective lumbar drain trial. Endocrine evaluation was performed at baseline and 1 and 3 months after shunt placement.

Results:
A total of 18 patients were enrolled (14 males, average age 62 years). Four patients were withdrawn because surgery was deferred or baseline labs were unobtainable. Of the remaining 14 patients who had baseline pituitary labs, 4 had evidence of possible pituitary dysfunction. Two patients exhibited low random cortisol levels but repeat morning cortisol or cortisol response following adrenocorticotrophic hormone (ACTH) cosyntropin stimulation, were within normal range. There were also 2 cases of abnormally low testosterone levels. In one of these 2 cases, testosterone levels improved after shunt placement.

Discussion:
Two out of 14 patients studied had evidence of pituitary dysfunction, namely hypogonadism. In only one of these 2 patients was there normalization of testosterone levels after shunt placement, suggesting NPH as a possible etiology. We conclude that NPH is rarely associated with pituitary dysfunction and was only observed in 7% of patients studied. As such, we recommend that pituitary screening should be based on relevant clinical signs and symptoms and is not indicated for all patients with NPH prior to surgical intervention.

Nothing to Disclose: TM, MB, APH

Pub # P2-402

Session Information POSTER SESSION: CLINICAL - Hypopituitarism (1:30 PM-3:30 PM)

Title Assessment of the Pituitary Function in Nasopharyngeal Carcinoma Patients Treated with Radiotherapy

Author String SH Ipekci, M Cakir, A Kiyici, M Artac
Selcuk University, Meram School of Medicine, Konya, Turkey; Selcuk University, Selcuklu School of Medicine, Konya, Turkey; Selcuk University, Meram School of Medicine, Konya, Turkey

Body Objective: High-dose radiotherapy, with good response rates, is used in the management of nasopharyngeal carcinoma. The hypothalamo-pituitary axis is a radiosensitive area in the central nervous system. Radiation induced hypopituitarism is an important late complication of cranial radiotherapy in children and adults. Hypopituitarism is associated with the total dose received and it worsens over time during the follow-up period. The purpose of this cross-sectional study was to evaluate the effects of radiotherapy on pituitary function in adult nasopharyngeal carcinoma patients.

Materials and methods: Pituitary function was evaluated in 30 patients (20 males - mean age 45.3±11.4 years, 10 females - mean age 45.1±5.9 years) after cranial radiotherapy for nasopharyngeal carcinoma. Somatotroph and corticotroph axes were assessed by insulin tolerance test while gonadotroph and thyroid axes were evaluated by basal pituitary and end organ hormone levels at 10-133 months after radiotherapy.

Results: At least one hormonal disorder was observed in 28 (93%) patients after radiotherapy. Twenty-six (87%) patients had one or more anterior pituitary hormone deficiencies. The rates of pituitary hormone deficiencies were 77% for growth hormone, followed by adrenocorticotrophic hormone (73%), thyroid-stimulating hormone (27%) and gonadotropins (7%). Hyperprolactinemia was present in 13 (43%) patients. Also primary gonadal deficiency was found in four males (13%) possibly due to previously used chemotherapeutic drugs. None of the patients developed diabetes insipidus or prolactin deficiency after radiotherapy. A negative correlation was noted between maximum cortisol response to hypoglycemia and the period after radiotherapy. None of our patients had secondary hypothyroidism and / or gonadotropin deficiency without adrenocorticotrophic hormone and / or growth hormone deficiencies.

Conclusions: Radiation-induced hypopituitarism is more common than expected in patients with nasopharyngeal carcinoma. The irreversible and progressive nature of radiation induced anterior pituitary hormone deficiencies validate evaluation of pituitary function in these subjects for timely diagnosis and treatment of hypopituitarism.

Nothing to Disclose: SHI, MC, AK, MA

Pub # P2-403

Session Information POSTER SESSION: CLINICAL - Hypopituitarism (1:30 PM-3:30 PM)

Title Pituitary Function in Type 2 Diabetic Men with Good or Poor Glycemic Control

Author String S Scaltriti, B Madeo, M Bertolini, S Romano, L Zirilli, ER Cavani, A Ballestrieri, C Carani, V Rochira
Department of Medicine, Endocrinology & Metabolism, Geriatrics, NOCSAE of Baggiovara, University of Modena & Reggio Emilia, Modena, Italy; Department of Medicine, Endocrinology & Metabolism, Geriatrics, NOCSAE of Baggiovara, University of Modena & Reggio Emilia, Modena, Italy; Bufalini Hospital, Cesena, Italy

Body **INTRODUCTION** Several data suggest that male hypogonadism occurs frequently in men with type 2 diabetes mellitus (T2DM) but underlying pathophysiological mechanism remains partially unknown. In order to investigate the short term effect of hyperglycaemic state on hypothalamic-pituitary-gonadal axis we prospectively studied 18 men with T2DM younger than 55 years at the time of first diagnosis of T2DM. We compared men with HbA1c \geq 9.0% with men with HbA1c \leq 9.0% at baseline at baseline when glycaemic control was wrong and after a short and long period of treatment when a good glycaemic control had been obtained.

METHODS Basal serum fasting glucose, insulin, fructosamine, HbA1c, total, HDL-, LDL-cholesterol, triglycerides, LH, FSH, total testosterone (T), estradiol and stimulated LH and FSH after a standard GnRH test.

RESULTS A positive percentage increase from baseline of T was observed in men with HbA1c [ge] 9% at baseline after glycaemic control had been achieved, while in men with HbA1c < 9% a minimal decrease of T was recorded, the mean percentage increase from baseline of group with HbA1c [ge] 9% being significantly higher (p=0.018), and it was associated with a positive trend of T/LH ratio and a positive percentage increase from baseline of the response of LH to GnRH infusion in terms of Area Under the Curve and peak.

CONCLUSION T secretion by Leydig cells could be impaired in poorly controlled T2DM men and both a better glycaemic control at baseline and/or an improvement of the glycaemic control are associated with a better gonadal function in T2DM men.

Nothing to Disclose: SS, BM, MB, SR, LZ, ERC, AB, CC, VR

Pub # P2-404
Session Information POSTER SESSION: CLINICAL - Hypopituitarism (1:30 PM-3:30 PM)
Title Acute Psychotic Illness as the First Manifestation of Hypopituitarism
Author String C Keshavamurthy, V Bellot, I Sachmechi
Queens Hospital Center/Mount Sinai School of Medicine, Jamaica, NY

Body

Objective

Report a case who presented with psychosis and hyponatremia and failed to respond to antipsychotics, fluids, fluid restriction, but responded to cortisol and thyroid hormone replacement.

Case

A 63 year old female with history of diabetes mellitus and dyslipidemia was hospitalized for acute psychosis. She was treated with antipsychotics, without improvement. She denied being a smoker, alcoholic or drug abuser. Review of systems was essentially negative. Physical exam: blood pressure 108/74, Pulse 66. She was afebrile and euvolemic. Labs revealed normal white blood cell count, with neutropenia and lymphocytosis. Sodium was 122 mEq/l (136 to 146). Rest of her electrolytes was normal. Her course of hospitalization was characterized by hypotensive episodes. Serum osmolality was 259 mosm/kg (275 to 295), urine sodium 40 mEq/ml, urine chloride 58 mEq/ml, urine osmolality 517 mosm/kg (50 to 1200). Serum cortisol was 3.1 micrograms/dl (0.7 to 22.4), thyroid stimulating hormone was 3.33 MIU/L (0.34 -5.6), Free T4 0.38 ng/dl (0.58 to 1.64). Thyroperoxidase and anti-thyroglobulin antibodies were negative. Cosyntropin test revealed serum cortisol of 2.9Ug/dl at 0 hours and 9 micrograms/dl at one hour. Follicular stimulating hormone was 0.68 Miu/ML, Insulin Growth Factor-1 was 22 ng/ml (13 to 73), prolactin was 9.28 ng/ml (2.74 to 19.64). CT brain and chest X-ray were normal. Magnetic Resonance Imaging of the brain revealed findings consistent with partial empty sella turcica. The patient was started on hydrocortisone and levothyroxine following which there was a drastic improvement in serum sodium and significant resolution of her psychosis.

Discussion

The exact mechanism by which psychosis occurs in individuals with hypopituitarism is still obscure, although it has been postulated that hypothyroidism, hypoglycemia and hypocortisolism may all have a role. Awareness of the pathognomonic findings of hypopituitarism and characteristic symptom complexes associated with hypopituitarism-related psychosis may lead to increased recognition of this condition prior to the onset of coma.

Testing for adrenal insufficiency and hypothyroidism should be part of the hyponatremic work-up, as the disorders respond promptly to hormone replacement.

Conclusion

In a patient presenting with acute psychosis, in the setting of electrolyte abnormalities, work up for hypopituitarism should be pursued if refractory to routine interventions.

Nothing to Disclose: CK, VB, IS

Pub # P2-405

Session Information POSTER SESSION: CLINICAL - Hypopituitarism (1:30 PM-3:30 PM)

Title Vertical Transmission of Hypopituitarism: Implications for Treatment during Pregnancy

Author String ED Pine-Twaddell, C Romero, S Radovick
Johns Hopkins University, Baltimore, MD

Body

Introduction: Patients with combined pituitary hormone deficiency may have preserved fertility.

Clinical Case: A woman with a history of growth hormone deficiency and central hypothyroidism gave birth to a term AGA male neonate, who was the result of a spontaneous pregnancy. At 15 weeks gestation, the mother's TSH = 0.058 uIU/mL, Free T4 = 0.8 ng/dL (<5th%). At 18 weeks, TSH was 0.067 uIU/mL, and levothyroxine dose was decreased by the obstetrician. At 27 weeks, free T4 was 0.44 ng/dL. The mother was unable to lactate in the postpartum period. At birth, the neonate had physical features of macrocephaly, fronto bossing, and a depressed nasal bridge with wide, anteverted nares. His hospital course was complicated by hypotonia, respiratory distress, and feeding intolerance. The neonatal laboratory evaluation showed a TSH = 0.98 uIU/mL, total T4 = 2.1 mcg/dL (8.2-16.6), undetectable GH, IGF-I, and IGFBP3, a normal cortisol level and normal gonadotropin surge. An MRI showed a normal pituitary gland. After initiation of levothyroxine in the first week, both tone and feeding tolerance improved, nevertheless, the patient developed mild-moderate sensorineural hearing loss, gross motor delay, and speech delay. Informed consent was obtained to analyze the patient's and mother's DNA for candidate genes required for normal pituitary development and associated with the development of hypopituitarism. The patient and his mother were both found to harbor a heterozygous R271W mutation in the homeodomain of POU1F1, which has previously been described as a dominant negative mutation causing GH, TSH and prolactin deficiency. Lack of sufficient placental transfer of thyroid hormone during the prenatal period has been shown to result in hearing loss and cognitive defects. Both mother and fetus were unable to synthesize sufficient thyroid hormone, which may be responsible for the patient's clinical presentation.

Clinical Lessons: This case underscores several important points in the management of women with hypopituitarism: 1) The importance of patients and clinicians both being aware of the differences in etiology, screening, and treatment of primary vs. central hypothyroidism; 2) The necessity of close monitoring of thyroid hormone status during pregnancy to prevent fetal sequelae of maternal hypothyroidism; and 3) the necessity of genetic screening of patients with combined pituitary hormone deficiency, so that prenatal genetic counseling may be an option for expecting parents.

Nothing to Disclose: EDP-T, CR, SR

Pub # P2-406

Session Information POSTER SESSION: CLINICAL - Hypopituitarism (1:30 PM-3:30 PM)

Title Treatment of Lymphocytic Hypophysitis with Panhypopituitarism: Improvement by High-Dose Methylprednisolone Pulse Therapy

Author String S Shen, W Chen, D Zhu, Y Bi, B Zhang
The Affiliated Drum Tower Hospital of Nanjing University Medical School, Nanjing, China; The Affiliated Drum Tower Hospital of Nanjing University Medical School, Nanjing, China

Body Background: Lymphocytic hypophysitis (LHy) is an uncommon disease that should be considered in the differential diagnosis of any nonsecreting pituitary mass, especially when occurring during pregnancy or postpartum. we outline a case of LHy with panhypopituitarism showing improvement of symptoms post-initiation of high dose methylprednisolone pulse therapy (HDMPT). Clinical case: A 30-year-old woman 6 months post-partum was admitted on July 24, 2009 with a headache. On February 24, 2009 she gave birth to her first child. She attempted to breast-feed but had little milk. the MRI performed in June,2009 revealed a large mass involving the sella and suprasellar region that measured 2.5 cm. The endocrinological evaluation revealed that panhypopituitarism. The patient was accepted a course of steroid treatment without improvement. She further complained of a severe polyuria and visual disturbance. On June 24, 2009, the patient was admitted for a transsphenoidal operation. A partial pituitary resection revealed lymphocytic hypophysitis. After two weeks, she described profound weakness, polyuria and polydipsia. Upon admission, the MRI gland showed a 1.9[times]1.4[times]1.1 cm sized, well-enhanced pituitary mass. The typical clinical manifestation, and the pictures of the MRI scan could be considered compatible with the diagnosis of LHy. w administered HDMPT (400mg methylprednisolone daily for two days, then 200mg daily for two days, 100mg daily for two days.). The patient's visual sensation became clear and her headache was improved. A pharmacological dose of prednisolone (30 mg daily) was initiated, and tapered gradually without recurrence of the disease on low-dose prednisolone. MRI taken three months later revealed marked mass reduction. No recurrence occurred during follow-up for 18 months.

Conclusion :LHy is a rare disorder that should be considered in the diagnosis of pituitary mass. The mass may extend to the suprasellar region. In addition, Lhy should be suspected in patients with hyperprolactinemia, hypopituitarism and cranial diabetes insipidus. Furthermore, we recommend the high dose methylprednisolone to treat it. It can resolve the pituitary mass and improve the endocrine dysfunction.

1. Rudolf A Kristof, Dirk Van Roost, Dietrich Klingmüller. Lymphocytic hypophysitis: non-invasive diagnosis and treatment by high dose methylprednisolone pulse therapy? J Neurol Neurosurg Psychiatry 1999;67:398-402

Nothing to Disclose: SS, WC, DZ, YB, BZ

Pub # P2-407

Session Information POSTER SESSION: CLINICAL - Hypopituitarism (1:30 PM-3:30 PM)

Title A Case of Postpartum Hyponatremia in Diabetes Insipidus

Author String A Chandrasekhar, S Soler, M Miller, G Gopalakrishnan
Alpert Medical School of Brown University, Providence, RI

Body **Background:** Knowledge of how pregnancy affects vasopressin (AVP) metabolism is essential for management of patients with pre-existing diabetes insipidus (DI). The placenta produces vasopressinase, a cystine aminopeptidase which inactivates AVP. Vasopressinase levels increase 1000-fold in pregnancy, peak at term, and decline by one month postpartum. Desmopressin (DDAVP) is the drug of choice for treatment of DI during pregnancy, and is resistant to degradation by vasopressinase. We present a case of iatrogenic hyponatremia in a pregnant subject with pre-existing DI on DDAVP.

Clinical Case: A 21 year-old female at 38 weeks' gestation underwent successful caesarean section. The patient's past medical history was significant for central diabetes insipidus diagnosed at age six secondary to infiltration of the hypothalamic-pituitary axis from Histiocytosis X. During pregnancy, her DDAVP dose was increased from one spray twice daily to four times daily. Peri-operatively, she received two liters of normal saline. On post-op day one, she took three sprays of DDAVP. On post-op day two, her serum sodium levels decreased from 134 to 122 over a 24-hour period. Laboratory values were as follows: serum osmolality of 248 mOsm (n 275-295), urine osmolality of 981 mOsm (n 50-1400), urine sodium of 38 (n 10-40 mEq/L), and urine specific gravity of 1.016 (n 1.010-1.020). The patient remained asymptomatic and was clinically euvoletic. She was placed on fluid restriction. Serum sodium normalized to 135 over a 36-hour period. Her DDAVP dose was decreased to one spray daily, and she was discharged.

Conclusion: We conclude that increased DDAVP and IV fluids during labor caused an excess of free water relative to sodium, resulting in severe hyponatremia post-partum. Frequent monitoring of serum and urine electrolytes is critical in the management of DI patients on DDAVP during labor and delivery. Vasopressinase causes increased AVP requirements ante-partum, and decreased requirements post-partum. Therefore, titration of DDAVP dosing during and after pregnancy may be necessary. The rate of metabolic clearance of DDAVP increases only minimally during pregnancy, supporting the idea that vasopressinase primarily affects degradation of endogenous AVP. Increased awareness of the potential consequences of excess DDAVP and IV fluid administration in pregnant subjects with DI is crucial for prevention and diagnosis of iatrogenic hyponatremia.

1) Iwasaki Y, Oiso Y, Kondo K. Aggravation of Subclinical Diabetes Insipidus During Pregnancy. *N Engl J Med.* 1991 Feb 21; 324(8):522-6.

2) Ananthakrishnan S. Diabetes insipidus in pregnancy: etiology, evaluation, and management. *Endocr Pract.* 2009 May-Jun;15(4):377-82.

3) Davison JM, Sheills EA, Philips PR, Barron WM, Lindheimer MD. Metabolic clearance of vasopressin and an analogue resistant to vasopressinase in human pregnancy. *Am J Physiol.* 1993 Feb; 264(2 Pt 2):F348-53.

Nothing to Disclose: AC, SS, MM, GG

Pub # P2-408

Session Information POSTER SESSION: CLINICAL - Hypopituitarism (1:30 PM-3:30 PM)

Title Diabetes Insipidus Management in High-Dose Methotrexate Therapy for Primary CNS Lymphoma

Author String MM Sarkar, E Chin
Charlie Norwood Veterans Administration Medical Center, Augusta, GA; Medical College of Georgia,
Georgia Health Science University, Augusta, GA

Body Introduction: Primary CNS lymphoma (PCNSL), a form of extranodal high-grade non-Hodgkin's B-cell neoplasm, is a rare cancer. Primary involvement of the sellar and parasellar region causing diabetes insipidus (DI) is even less common. PCNSL chemotherapy with high dose methotrexate (hdMTX) requires intravenous fluids and high urine output challenging DI management.
Clinical case: A 60 year-old man presented with acute blurred vision, confusion and increased thirst. Initial sodium was 142 and rapidly rose to 155 (n135 - 145 mEq/L) due to altered mental status and impaired thirst sensation. Studies were consistent with DI and panhypopituitarism (urine osmolality 186 mOsm/L, plasma osmolality 325 (n 285 - 295 mOsm/L), peak cortisol 4.5 mcg/dL 60 minutes after cosyntropin 0.25 mg, TSH 1.53 (n 0.5 - 5.4 mIU/mL), free T4 <0.50 (n 0.61 - 1.1 ng/dl). Hydrocortisone, levothyroxine and dDAVP were started. MRI showed a 20 x 22 x 12 mm third ventricular floor mass with well-defined margins and intense enhancement producing compressive deformity and anterior displacement of the optic chiasm. Lumbal puncture revealed hypercellular cerebrospinal fluid with numerous mature lymphocytes, a few monocytes and atypical hemato-lymphoid cells. Flow cytometry showed T-cells and few polyclonal B-cells with no aberrant immunophenotype. Brain biopsy showed atypical lymphoid cells and foamy cells with reactive gliosis consistent with non-Hodgkin's lymphoma, diffuse large B-cell type diagnostic of PCNSL. A hdMTX and leucovorin rescue chemotherapeutic regimen was selected and intravenous fluid hydration and urine alkalization were initiated to prevent methotrexate (MTX) induce renal dysfunction. Fluid balance, serum creatinine, serum sodium, urine pH and serum MTX levels were closely monitored. Increased dDAVP administration during hdMTX administration was necessary to prevent hypernatremia.
Conclusion: DI management is complicated by the use of hdMTX which requires aggressive high volume hydration with sodium bicarbonate for urine alkalization to maintain sufficient urine output and prevent renal failure. Drug induced nephrogenic DI due to cytotoxic agents is rare and MTX may impair dDAVP action^{1,2}. Panhypopituitarism and DI may persist despite remission. Judicious dDAVP dosing to maintain fluid balance and a normal serum sodium level in patients with altered thirst mechanisms who require intravenous fluids and high urine flow poses a challenge to effective DI management.

(1) Fernandez-Espertero MC, et al., Rheumatology 2002;41:3233-234.

(2) Lau KK, et al., J Oncol Pharm Pract 2005;11:31-33.

Nothing to Disclose: MMS, EC

Pub # P2-409

Session Information POSTER SESSION: CLINICAL - Hypopituitarism (1:30 PM-3:30 PM)

Title High Risk of Central Adrenal Insufficiency in GH-Deficient Long-Term Survivors of Childhood Acute Lymphoblastic Leukemia

Author String C Follin, K Link, C Moell, T Wiebe, EM Erfurth
Clinical Sciences, Lund University, Lund, Sweden; Clinical Sciences, Lund University, Lund, Sweden

Body
Introduction: Acute lymphoblastic leukaemia (ALL) is the most common childhood malignancy and accounts for 25% of all childhood cancer. The survival rate is now 85% which emphasizes the importance of long-term treatment complications. ALL patients treated with cranial radiotherapy (CRT) and chemotherapy are particularly at risk for growth hormone deficiency (GHD), but little is known about central adrenal deficiency. Partial ACTH deficiency is often asymptomatic, but it may lead to deleterious consequences during stress.
Methods: Out of 44 (21 women) ALL patients (median 25 years, range 19-32), treated with 24 Gy CRT (18-24) and chemotherapy, 37 were tested with an insulintolerance test (ITT) to evaluate GH and cortisol secretion. All were GHD based on the GH response (<3 ug/L). The normal cortisol cut-off level was [ge] 500 nmol/L. Basal serum ACTH- and cortisol-levels were investigated in all 44 ALL patients and 44 matched population controls.
Results: 14 out of 37 (38%) ALL patients had subnormal cortisol response to an ITT (257-478 nmol/L) while there was no significant difference in basal serum cortisol levels between 44 patients and controls ($P > 0.3$). ALL patients had significantly lower serum ACTH levels compared to their controls ($P = 0.04$), being significantly lower among the women ($P = 0.03$), only. There was a significantly positive correlation between peak serum cortisol after ITT and basal cortisol ($P < 0.001$, $r = 0.6$), and the GH peak after ITT ($r = 0.3$, $P = 0.04$).
Conclusion: 38% of former ALL patients with GHD, treated with CRT, have central adrenal insufficiency 20 years after ALL diagnosis. About 500 ALL survivors have been subjected to this therapy in Sweden (pop. 9 millions), and with the corresponding numbers in other countries. This is of great concern, particularly as GHD masks the presence of a hidden central adrenal insufficiency.

Disclosures: EME: Scientific Board Member, Speaker, Eli Lilly & Company. Nothing to Disclose: CF, KL, CM, TW

Pub # P2-410

Session Information POSTER SESSION: CLINICAL - Hypopituitarism (1:30 PM-3:30 PM)

Title Central Adrenal Insufficiency Due to Epidural and Facet Joint Injections and Chronic Opioid Use

Author String SL Liu, TL Paul, SHM Van Uum
Schulich School of Medicine & Dentistry, University of Western Ontario, London, Canada

Body

Background: Adrenal insufficiency (AI) is an important, potentially life-threatening condition. Central AI can be difficult to recognize and diagnose, especially in patients with chronic pain in whom systemic absorption of locally injected steroids and hypothalamic-pituitary-adrenal axis suppression by chronic opioids may be risk factors for development of AI.

Clinical Case: A 45 year old woman was admitted to hospital with severe epigastric pain, nausea and vomiting. Hypotension was not present. Multiple investigations did not yield an etiology. Her symptoms slowly improved after IV fluids, anti-emetics and analgesics. She reported multiple similar episodes requiring hospital admission since 2004, occurring every 6 to 12 months. Her past medical history was complex, and included chronic back pain from motor vehicle accidents in 2001 and 2003, treated with a fentanyl patch 175 mcg/hr q48 hours and oxycocet 325 mg/5 mg q.i.d. She had never received oral glucocorticoids. She noted recurrent intermittent orthostatic dizziness and a 7 year history of oligomenorrhea.

Physical exam did not show any features of adrenal insufficiency. An a.m. cortisol was low at 56 nmol/L (119-618). Repeat 8 AM cortisol 220 was nmol/L with ACTH 7.11 pmol/L (1.98-12.47). Further hormonal evaluations demonstrated mild hypogonadotropic hypogonadism and mild decreased IGF-1. We suspected central AI and started hydrocortisone, resulting in significant clinical improvement. Repeat lab work (off hydrocortisone) confirmed central AI. CT pituitary did not show any pituitary abnormality, so chronic opioid use was considered to be the likely cause.

At a follow-up visit she indicated having received intermittent epidural and facet joint injections with methylprednisolone over the past 5 years. Thus, we thus speculate that her central AI is due to either systemic absorption of her local glucocorticoid injections, or chronic opioid use, or a combination of both.

Conclusion: Central AI, though not common, can develop in patients being treated for chronic pain, and several mechanisms may play a role. Heightened awareness of this rare complication is required, especially in symptomatic chronic pain patients receiving opioids and/or local steroid injections.

Nothing to Disclose: SLL, TLP, SHMVU

Pub # P2-411

Session Information POSTER SESSION: CLINICAL - Hypopituitarism (1:30 PM-3:30 PM)

Title Low-Dose Hydrocortisone (HC) Replacement Therapy Is Associated with Improved Bone Remodeling Balance in Hypopituitary Subjects

Author String LA Behan, G Kelleher, MJ Hannon, JJ Brady, B Rogers, W Torney, D Smith, CJ Thompson, MJ McKenna, A Agha
Beaumont Hospital, Dublin, Ireland; Beaumont Hospital, Dublin, Ireland; St Vincent's University Hospital, Dublin, Ireland; St Vincent's University Hospital, Dublin, Ireland

Body The effect of commonly used glucocorticoid replacement regimens on bone health in hypopituitary subjects is not well known. We aimed to assess the effect of 3 hydrocortisone (HC) replacement dose regimens on bone turnover in this group.
10 hypopituitary men with severe ACTH deficiency were randomised in a crossover design to 3 HC dose regimens, Dose A (20mg mane, 10mg tarde), Dose B (10mg twice daily) and Dose C (10mg mane, 5mg tarde). Following 6 weeks of each regimen participants underwent fasting sampling of bone turnover markers. Data from matched controls were used to produce a Z score for subject bone formation and resorption marker and to calculate the bone remodeling balance (formation Z score-resorption Z score) and turnover index ((formation Z + resorption Z)/2). A positive bone remodeling balance with increased turnover is consistent with a favourable bone cycle. Data are expressed as median (range).
The Pro Collagen Type 1 Peptide (PINP) bone formation Z-score was significantly increased in Dose C, (1.805 (-0.6-10.24)) compared to Dose A (0.035 (-1.0-8.1)) $p<0.05$ while there was no difference in the C-terminal crosslinking telopeptide (CTx) resorption Z score. The bone remodeling balance was significantly lower for dose A -0.02 (-1.05-4.12) compared to dose C 1.13 (0.13-6.4) ($p<0.05$). Although there was a trend to an increased bone turnover index with the lower dose regimen, this was not statistically significant.
Low dose HC replacement (10mg mane/5 mg tarde) was associated with increased bone formation and improved bone remodeling balance which is associated with a more favourable bone cycle. This may have a long term beneficial effect on bone health.

Nothing to Disclose: LAB, GK, MJH, JJB, BR, WT, DS, CJT, MJM, AA

Pub # P2-412

Session Information POSTER SESSION: CLINICAL - Hypopituitarism (1:30 PM-3:30 PM)

Title Is Low Prolactin an Indicator of True GH Deficiency in Adults?

Author String R Alagha, C Murray, L Kingdon, S Ezzat
University Health Network, Toronto, Canada

Body

Background: Growth hormone (GH) secretion is pulsatile requiring provocative dynamic testing such as the insulin tolerance test to prove true hormone deficiency. Due to lack of wide availability and the potentially harmful effects of the insulin tolerance test (ITT), a static surrogate hormone marker of GH deficiency would be clinically helpful.

Aim: To determine the utility of a low baseline prolactin level as a predictor of subnormal GH responses in insulin tolerance testing

Methods and Results: A retrospective chart review of patients with known pituitary dysfunction was carried out in a tertiary care University centre. Prolactin levels drawn at the time of the ITT. The prolactin, IGF-1 and ITT results of 33 consecutive patients with proven GH deficiency. Eleven age-matched controls with suspected but proven GH sufficiency served as controls. Differences in levels and responses were compared using Fischer's Exact test.

All patients were adults with suspected hypopituitarism. The pituitary disorders included mostly patients with histologically-proven non-functioning pituitary adenomas. However other diagnoses also included: Pit-1 mutation (n=2), Cushing's disease (n=1), congenital pituitary dysplasia (n=1), autoimmune hypophysitis (n=2), craniopharyngioma (n=3), optic glioma (n=1), and medulloblastoma (n=1). Patients with GH deficiency as defined by a peak GH <5 ug/L during ITT testing displayed lower prolactin levels (5.2 ± 3.2 ug/L) compared with those who demonstrated normal GH responses whose prolactin levels were 11.1 ± 3.9 ug/L ($p=0.0006$). Of note, however, nearly half (16/33) patients with true GH deficiency also had a low (<2SD) baseline IGF-1.

We conclude that in the clinical context of possible hypopituitarism, a low basal prolactin serves as an additional surrogate marker of true GH deficiency. Larger prospective studies are currently underway to examine this feature.

Nothing to Disclose: RA, CM, LK, SE

Pub # P2-413

Session Information POSTER SESSION: CLINICAL - Hypopituitarism (1:30 PM-3:30 PM)

Title Growth Hormone Replacement Therapy in Elderly Growth Hormone-Deficient Patients: A Systematic Review

Author String NE Kokshoorn, NR Biermasz, F Roelfsema, JWA Smit, AM Pereira, JA Romijn
Leiden University Medical Center, Leiden, Netherlands

Body
Context: Recombinant human growth hormone (rhGH) is indicated for the treatment of adult subjects with growth hormone deficiency (GHD). However, conflicting data are available on the efficacy of rhGH treatment in elderly GHD patients.
Objective: To assess the efficacy of rhGH treatment in elderly GHD subjects
Methods: We searched PubMed, Cochrane Library, Web of Science, and EMBASE.
Study selection: Eligible studies included GHD patients, aged > 60 years, treated by rhGH. Data extraction was performed by two reviewers independently.
Results: We found 11 eligible studies with a total of 534 patients. Only 2 studies had prospective, randomized placebo-controlled study designs with a duration of rhGH treatment of 6 (n=15) and 12 months (n=62), respectively. Treatment with rhGH decreased total and LDL-cholesterol levels by 4-8% and 11-16%, respectively, but did not alter HDL or triglyceride (TG) levels. RhGH did not affect body mass index, but decreased waist circumference (by~3 cm) and waist/hip ratio. RhGh did not consistently affect blood pressure or bone mineral density. RhGH increased lean body mass by 2-5 % and decreased total fat mass by 7-10 % in 4 studies, but did not affect body composition in 2 other studies. RhGH consistently improved quality of life parameters reflected in AGHDA-scores. There are no explicit data on elderly GHD patients aged > 80 yrs.
Conclusion: The effects of rhGH replacement in elderly subjects with GHD are relatively limited. In addition, there are no data on the efficacy and safety of rhGH treatment in GHD octogenarians.

Nothing to Disclose: NEK, NRB, FR, JWAS, AMP, JAR

Pub # P2-414

Session Information POSTER SESSION: CLINICAL - Hypopituitarism (1:30 PM-3:30 PM)

Title Growth Hormone Treatment Improves Non-Alcoholic Fatty Liver Disease (Nafld) in Patients with Adult Growth Hormone Deficiency

Author String M Kurimoto, I Fukuda, S Tanaka, Y Yamakado, T Muraoka, K Takano, N Hizuka
Tokyo Women's Medical University, Tokyo, Japan

Body **Background:** Adult growth hormone deficiency (GHD) is frequently associated with metabolic disorders, such as visceral obesity, abnormal lipid profiles and insulin resistance. Recently, we demonstrated that liver dysfunctions with nonalcoholic fatty liver disease/steatohepatitis (NAFLD/NASH) are frequently observed in patients with adult GHD.
Objective: We investigated whether GH replacement therapy improves the liver dysfunction as well as other metabolic profiles in patients with adult GHD.
Subjects and Methods: Forty-eight patients with adult GHD (M/F 23/25, 18-66 yrs) have been treated with GH for more than 1year. We analyzed the medical records of these patients to investigate liver function, liver findings by ultrasonography and metabolic complications revealed at baseline and after the GH treatment.
Results: Serum AST and ALT levels at baseline were elevated in 16 (33%) and 24 (49%) patients, respectively. After the GH treatment, serum AST and ALT levels decreased and were still elevated only in 4 (8%) and 9 (18%) patients, respectively. We investigated the liver findings by ultrasonography at baseline in 28 patients, fatty liver was detected in 20 patients. Thirteen of the 20 patients who had fatty liver at baseline were reexamined by ultrasonography after GH treatment, we observed that fatty liver disappeared in 8 patients. As these patients had neither heavy drinking habit (alcohol intake less than 100g of ethanol per week) nor any liver disease (e.g., viral infection, autoimmune hepatitis), these patients were diagnosed as NAFLD. For dyslipidemia, high LDL-cholesterolemia, low HDL-cholesterolemia and hypertriglyceridemia were observed in 16 (32%), 11 (22%) and 30 (61%) of the patients at baseline, respectively. After GH treatment, high LDL-cholesterolemia improved, but, serum levels of HDL-cholesterol and triglyceride did not significantly change.
Conclusion: These data suggest that GH treatment improve the liver dysfunction with NAFLD in adult GHD.

Nothing to Disclose: MK, IF, ST, YY, TM, KT, NH

Pub # P2-415

Session Information POSTER SESSION: CLINICAL - Hypopituitarism (1:30 PM-3:30 PM)

Title The Maintenance Dose of Growth Hormone Therapy and Its Efficacy in Japanese Patients with Adult GH Deficiency

Author String I Fukuda, M Kurimoto, K Takano, S Tanaka, Y Yamakado, T Muraoka, R Oba, C Sekiyama, N Hizuka
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Body [Introduction] In Japan, GH therapy for adult GH deficiency was approved in 2006. Since then, our experience of GH therapy for Japanese adults with GH deficiency has been gradually accumulating. In Japanese guideline for the management of patients with adult GH deficiency, the starting dose of GH is recommended as 3[μ]g/kg/day and then, the dose should be titrated individually based on clinical response and serum IGF-I levels. In this study, we studied the maintenance dose of GH and its efficacy in Japanese patients with adult GH deficiency in a clinical practice. [Subjects and Methods] The maintenance dose of GH and recent serum IGF-I levels were investigated in 25 patients with adult GH deficiency (M/F 12/13, 21-60 years old, childhood onset/adulthood onset 19/6). All patients had received GH for at least 6 months and were in a stable condition. [Results] The median maintenance dose in 25 patients was 0.3mg/day (range: 0.1 - 0.6), 4[μ]g/kg/day (range: 1-9), respectively. During GH therapy, IGF-I levels were within normal range in 22 of 25 patients (M/F 12/10). GH dose of these 22 patients was 4[μ]g/kg/day (range: 1-7) in men and 4[μ]g/kg/day (range: 3-9) in women. There was no gender difference in the maintenance dose, although low serum IGF-I levels during GH therapy was observed in 3 women but not in men. During GH therapy, we observed improvement of QOL and non-alcoholic fatty liver disease in the patients. For dyslipidemia, anti-hyperlipidemia agents were combined with GH therapy in 16 patients but discontinuation of anti-hyperlipidemia agent was possible in only one patient. [Discussion] In general, women require higher dose of GH compared with men. We could not observe a gender difference in maintenance dose of GH in this study, although normal IGF-I level was not achieved in 3 women. We observed the improvement of serum IGF-I levels and clinical symptoms even in patients with adult GH deficiency who received relatively lower dose of GH (4[μ]g/kg/day). Further study is required to assess whether effects of replacement could be sustained in the long term by those maintenance dose of GH.

Nothing to Disclose: IF, MK, KT, ST, YY, TM, RO, CS, NH

Pub # P2-416

Session Information POSTER SESSION: CLINICAL - Hypopituitarism (1:30 PM-3:30 PM)

Title GH-Dependent Growth of a Pilocytic Astrocytoma in a Girl Receiving Hormonal Treatment for Tumor-Induced Hypopituitarism: IGF Receptor Status of the Tumor Tissue

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Body Introduction: Comparative studies from neurooncology centers and data analyses of large pharmacoepidemiological databases do not suggest that the incidence of tumor recurrence in children with brain tumors treated with growth hormone (GH) is generally increased. However, if tumor remnants are present it is recommended to withhold GH treatment until stable disease can be assumed. In the context of GH therapy, stable disease is often defined as lack of tumor progression over a time period of 1-2 years. A few patients are described, where expansion of CNS tumors coincided with GH therapy. In these reports information on the receptor status of the tumors is scarce. Here we report the IGF receptor status determined by immunohistochemical studies of a GH-dependent pilocytic astrocytoma in a girl. Clinical Case: A 4.5 year-old girl presented with strabismus, loss of vision of the right eye, and a history of night-time headaches, abdominal pain and vomiting over a 21-month period. MRI studies revealed a tumor (volume 55 ml) originating from the optic chiasm, extending to the frontobasal area, the mesencephalon and the interpeduncular cistern. Neurosurgically, a partial resection was achieved. Histology revealed a pilocytic astrocytoma (WHO grade I). Postoperatively, the girl developed central diabetes insipidus, hypocortisolism, and hypothyroidism and was treated accordingly. At age 6.3 yrs, the girl's height had decreased from a position on the 90. to the 25. centile (height velocity 2.3 cm/yr, <<3. centile). GH deficiency was confirmed (maximal GH 1.0 ng/mL on arginine infusion, 2.1 ng/mL after clonidine) and GH therapy (0.025 mg/kg/d) was started. Height velocity increased to 10.0 cm/yr, low IGF-I serum concentration returned to the normal range. Unexpectedly, after 1.1 yrs of GH therapy tumor size increased significantly. GH was discontinued, then statural growth declined, and tumor growth subsided. A similar coincidence between statural and tumor growth occurred during two further treatment courses with GH, so that no further attempts of GH treatment were made. In vitro Studies: Immunohistochemical staining against IGF-I receptor revealed cytoplasmatic immunopositivity in more than 85 % of the tumor cells. Conclusion: This patient represents a case of GH-dependent tumor growth coinciding with statural growth. Immunohistochemical positivity of the tumor tissue for the IGF-I receptor is compatible with a role of GH-induced IGF-I for tumor growth in this patient.

Disclosures: BPH: Scientific Board Member, Pfizer, Inc.; Speaker, Pfizer, Inc.; Speaker Bureau Member, Novo Nordisk. Nothing to Disclose: RW, BS, KS, AE, KK

Pub # P2-417

Session Information POSTER SESSION: CLINICAL - Hypopituitarism (1:30 PM-3:30 PM)

Title Effect of Growth Hormone as Add-On Treatment in Severe Fibromyalgia Syndrome: Results from the IIIb, CT27560 Placebo-Controlled, Multicenter Trial

Author String G Cuatrecasas, MJ Gonzalez, C Alegre, J Fernandez-Sola, FJ Garcia-Fructuoso, G Sesmilo, M Lage, E Granados, M Puig-Domingo
CM Teknon and C Sagrada Familia, Barcelona, Spain; Institut Universitari Dexeus, Barcelona, Spain; Hospital Clinic Universitari, Barcelona, Spain; CIMA Clinic, Barcelona, Spain; Institut Universitari Dexeus, Barcelona, Spain; Complejo Hospitalario Universitario Santiago Compostela, Santiago de Compostela, Spain; Merck SL, Madrid, Spain; Hospital Universitari Germans Trias i Pujol, Barcelona, Spain

Body
Introduction: Functional GH deficiency has been described in Fibromyalgia (FM)(1). The efficacy of GH as add-on treatment has been suggested in small studies (2,3), but little is known in larger and homogeneous populations.
Design: 120 patients were enrolled in a multicenter, randomized, placebo-controlled trial for 18 months (NCT00933686). FM impact questionnaire (FIQ) >75 (severe), duration of FM >18 months, and stability of the standard therapy (amitriptyline+opioids+SSRI) >12 months were required. Adult GH deficiency and insensitivity were ruled out with an ITT test and IGF1-generation test, respectively. In the first 6 months (blind-phase), Group A received 0.006 mg/kg/d of GH sc (further titrated based on IGF-1 levels) and group B received sc placebo, both added to standard therapy. Placebo arm was then switched to GH from 6-12 months (open-phase), and a follow-up study from 12-18 months was carried-on after GH withdrawal. Number and intensity of tender points, FIQ and subscales, Quality of Life test (EQ5D) with visual analogic scale (VAS) were evaluated at different time-points.
Results: At 12 months, 53% of the patients in group A (GH continuously) reached < 11 positive tender points (cut-off for diagnosis) versus 33% in group B (6 months of GH) (p<0,05). Significant improvements were also seen comparing FIQ (p=0,01), subscales (p<0,05) and EQ5D (p<0,05). In group A, 56,5% patients reached >30% improvement in VAS and 39,1% >50%, compared to 36.7% and 22.4% respectively in group B. Medication withdrawal in the extension study phase worsened all the scores within each group from the 1st month of follow-up (p<0,05). Carpal tunnel syndrome was the most prevalent related adverse event (17%) and no discontinuations were seen.
Conclusions: This is the largest and longest placebo-controlled trial performed so far in FM. Added GH is comparable to some labelled drugs in term of pain reduction, FIQ and VAS and further shows a sustained pattern of action.

1.-Cuatrecasas G, Gonzalez MJ, Alegre C, Sesmilo G, Fernandez-Sola J, Casanueva FF, Garcia-Fructuoso F, Poca-Dias V, Izquierdo JP, Puig-Domingo M. High prevalence of growth hormone deficiency in severe fibromyalgia syndromes. J Clin Endocrinol Metab. 2010; 95(9):4331-7.
2.-Bennett RM, Clark SC, and Walczyk J. A randomized, double-blind, placebo-controlled study of growth hormone in the treatment of fibromyalgia. Am J Med 1998;104:227-31.
3.-Cuatrecasas G, Riudavets C, Guell MA, Nadal A. Growth hormone as concomitant treatment in severe fibromyalgia associated with low IGF-1 serum levels. A pilot study. BMC Musculoskelet Disord. 2007; 30(8):119.

Sources of Research Support: Grant for clinical trial, from Merck SL, Spain.

Disclosures: GC: Study Investigator, Merck BV. MJG: Study Investigator, Merck BV. CA: Advisory Group Member, Merck BV. JF-S: Advisory Group Member, Merck BV. FJG-F: Study Investigator, Merck BV. GS: Study Investigator, Merck BV. ML: Study Investigator, Merck BV. EG: Employee, Merck BV. MP-D: Advisory Group Member, Merck BV.

Pub # P2-418

Session Information POSTER SESSION: CLINICAL - Hypopituitarism (1:30 PM-3:30 PM)

Title Sustained-Release Recombinant Human Growth Hormone Improved Body Composition and Quality of Life in Adults over 50 Years Old with Somatopause

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Body
Background: Treatment with recombinant human GH (rhGH) can help the elderly experiencing somatopause and the resultant metabolic impairment obtain partial recovery. However, aged adults suffer inconvenience from daily injection of existing rhGH.
Objectives: To evaluate the effects, safety, and compliance of weekly administered low dose of sustained-release rhGH (SR-rhGH) in aged adults with somatopause.
Design: This is a 26-week prospective, single-arm, multicenter pilot study.
Intervention/Participants: A total of 38 subjects, aged [ge] 50 years were enrolled and each received 2 mg of SR-rhGH for 26 weeks.
Results: The mean baseline IGF-1 level of 123.4 ± 41.6 ng/ml increased to 174.8 ± 59.6 ng/ml at week 4, and the level was maintained for the remainder of the study period. At week 26, an average lean body mass increased by 0.45 kg, waist circumference reduced by 1.06 cm, and the quality of life (QoL) was significantly improved ($P < 0.01$ in each index). Serum levels of biochemical markers of bone resorption and formation simultaneously increased. An estrogen substitute in women attenuated the beneficial effects of SR-rhGH on body composition and metabolic indices. There was no significant change in the body fat distribution or fat mass. Adverse events included pruritus (10.5%), arthralgia (5.3%), and edema (5.3%), but their symptoms were well tolerable.
Conclusions: Body composition and quality of life can be restored in part by the replacement of low dose SR rhGH for 26 weeks in patients with somatopause without significant adverse effects.

Nothing to Disclose: HS, JWH, YSC, SWK, EJJ, DK

Pub # P2-419

Session Information POSTER SESSION: CLINICAL - Hypopituitarism (1:30 PM-3:30 PM)

Title High Prevalence of Sleep Disturbances and Psychosocial Impairment in Irradiated Brain Tumor Survivors Despite Stable Endocrine Replacement Therapy

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Body

Objective: Since the middle of the twentieth century, radiotherapy has greatly improved survival rates of brain tumour patients. Consequently, more and more childhood brain cancer patients have reached adulthood. In the wake of this medical progress, new problems have become manifest: Subsequent systematic research has highlighted that central nervous system radiotherapy may have serious harmful long-term effects such as radiation-induced secondary tumours, psychosocial and endocrine impairments. Knowledge about such potential late sequelae is crucial in the follow-up and evaluation of treatment effects of long-term survivors. It was the aim of the present study to obtain systematic data on the long-term psychosocial impairments of adult patients after cranial irradiation with diagnosed endocrine failure on hormone replacement therapy in a specialised endocrine unit.

Design/Methods: A set of questionnaires assessing quality of life (QoL), depression, daytime sleepiness, sleep and psychological distress and ability to work was mailed to all patients treated in a specialized single centre late-effects clinic who had received cranial irradiation at least 36 months ago and were on stable hormone replacement, including growth hormone. QoL was assessed using the QoL-AGHDA and SF-36 questionnaire. The BDI-II was used to evaluate depression, and sleep disturbances and daytime sleepiness were assessed by the PSQI and the ESS, respectively. The WPAI-GH was used to measure capability and impairment at work.

Results: 54/197 questionnaires were returned at the first data close and analyzed. QoL in the QoL-AGHDA was severely diminished in 51% of the patients. Impaired mental QoL in the SF-36 was evident in 22% and 40% showed depressive symptomatology in the BDI-II. Sleep disturbances and daytime sleepiness were prominent with 35.2% of all investigated patients qualifying for specialist sleep advice according to the ESS.

Conclusion: Despite stable hormone replacement, brain-irradiated patients suffer from a high degree of psychosocial impairment, including reduced QoL, depression and, importantly, sleep disturbances. The dominant sleep disturbances observed were surprising and warrant intensive screening in this group of patients. The results of this study translate directly into clinical recommendations to screen for and treat psychosocial impairments in addition to endocrine replacement therapy.

Nothing to Disclose: IK-A, RA, RS, GB

Pub # P2-420

Session Information POSTER SESSION: CLINICAL - Hypopituitarism (1:30 PM-3:30 PM)

Title Epidemiologic Features and Predictors of Mortality in a Cohort of Adult Patients with Hypopituitarism

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Body INTRODUCTION: Hypopituitarism is associated to higher prevalence of cardiovascular risk factors and premature death. Some clinical characteristics have been associated with a worse prognosis. We review a retrospectively a large series of adult patients with hypopituitarism analyzing its prevalence, association with cardiovascular risk factors and mortality.

PATIENTS AND METHODS: 180 adult hypopituitary patients (58.1% females) from a population of 405218 inhabitants, followed for 10 years (prevalence 44.4 cases/100000). 83 cases were diagnosed during the period studied (Incidence: 2.05 cases /100000, year). Mean age at diagnosis was 45.2 ± 21.2 years (3-81).

RESULTS: The most frequent cause of hypopituitarism was a pituitary tumour and/or its treatment (80 cases, 44.6%), most of them non-functioning tumours (45 cases, 56.2%). 75% were macroadenomas (mean size 27.5 ± 11.3 mm, 4-51). 82 patients (45.6%) underwent surgery and 48 (26.7%) were irradiated. Pituitary deficiencies on follow-up were: FSH/LH (78.9%), TSH (71.1%), ACTH (59.4%), GH (55%) and ADH (18.9%). The degree of hypopituitarism (number of affected axes) was associated with radiotherapy ($p < 0.01$) but not with the aetiology of hypopituitarism or surgery. 16% of patients had diabetes, 34% high blood pressure, 38% hyperlipidemia, 16% cardiovascular disease and 54% obesity.

Seven patients (3,9%) died during the follow-up (48.1% females). It was found an association between mortality and radiotherapy ($p < 0.001$), hypopituitarism degree ($p < 0.01$), glucocorticoids doses ($p = 0.02$), diabetes ($p < 0.001$), hypertension ($p < 0.001$), hyperlipidemia ($p = 0.02$) and previous cardiovascular disease ($p = 0.03$).

CONCLUSION:
Prevalence of hypopituitarism in our general population is 44.4/100000 inhabitants, most of cases due to a pituitary tumour or its treatment. Cranial radiotherapy, the hypopituitarism degree, high substitutive steroid doses and some cardiovascular risk factors as diabetes, hypertension and hyperlipidemia are related to the incidence of mortality.

Nothing to Disclose: EF-R, IB, FFC

Pub # P2-421

Session Information POSTER SESSION: CLINICAL - Hypopituitarism (1:30 PM-3:30 PM)

Title Pituitary Dysfunction after Long-Term Follow-Up in Adult Patients after Cranial Radiotherapy for Non-Pituitary Tumors

Author String NE Kokshoorn, NM Appelman-Dijkstra, KJ Neelis, NR Biermasz, JWA Smit, AM Pereira
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Body
Background: Cranial radiotherapy for non-pituitary tumors may cause hypopituitarism. However, the reported prevalence of hypopituitarism varies between studies and structured endocrine assessments are not incorporated in the long-term follow-up.
Aim of the study: To evaluate pituitary function in patients irradiated for non-pituitary tumors at our center.
Patients and Methods: Systematic chart review of all patients treated with irradiation at our center for non-pituitary cerebral or nasopharyngeal tumors and hematological malignancies with available data on endocrine evaluation.
Results: Fifty-five patients, treated for intracerebral tumors (n=24), extracerebral tumors (n=14), hematological malignancies (n= 14), nasopharyngeal cancer (n=2), and cerebral metastasis (n=1) were identified. Median age at radiotherapy was 20(3-74) yrs and median radiation dose 54(7.5-69.8) Gy. The endocrine evaluation included basal morning hormone sampling and stimulation of GH and cortisol secretion with either ITT (n=36), metyrapone (n=3), combined GHRH/Arginine (n=4) CRH (n=11), and/or ACTH (n=7). After a median follow-up of 11.5 (0.5-38) yrs, any hypopituitarism was present in 32/55(58%) of cases. The prevalence of GHD was 45% (23/51). ACTH-deficiency was present in 37% (19/51), TSH-deficiency in 25% (14/55), and LH/FSH-deficiency in 31% (17/55) of the patients. Hyperprolactinemia was present in 31% (17/54) of the patients. A higher irradiation dose (>50 Gy) was associated with a significantly higher prevalence of hypopituitarism.
Conclusion: After long-term follow-up, hypopituitarism is prevalent in adult patients after cranial radiotherapy for non-pituitary tumors. Therefore, all patients treated by cranial radiotherapy should have structured periodical assessment of pituitary functions.

Nothing to Disclose: NEK, NMA-D, KJN, NRB, JWAS, AMP

Pub # P2-422

Session Information POSTER SESSION: CLINICAL - Hypopituitarism (1:30 PM-3:30 PM)

Title Immune Therapy for Metastatic Melanoma with Ipilimumab Is Associated with Anterior Pituitary Hormone Deficiencies

Author String L Min, A Vaidya, C Becker
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Body Ipilimumab is a fully human monoclonal antibody against Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4). CTLA-4 counterbalances immune cell activation by inhibiting proliferation of T lymphocytes and secretion of interleukin 2 (1). In patients with metastatic melanoma, ipilimumab increases survival time and induces complete remission in some patients (2); however, immune-related adverse events have been reported (3-4). Herein, we describe six patients with advanced melanoma who received ipilimumab therapy and developed hypophysitis with manifestations of pituitary hormone deficiencies. All six patients were referred for either symptomatic adrenal insufficiency or abnormal thyroid function testing. The onset of symptoms and/or biochemical evidence of pituitary hormone deficiency occurred following 6-12 weeks of ipilimumab therapy. Testing for pituitary hormone deficiencies was undertaken by measuring AM cortisol with corticotropin levels, thyrotropin with thyroxine levels, gonadotropins with sex hormones, and growth hormone with insulin like growth factor 1. All six patients demonstrated clinical and biochemical evidence of secondary adrenal insufficiency and were placed on corticosteroid therapy. All six patients also displayed clinical and biochemical evidence of central hypothyroidism; three already had primary hypothyroidism prior to ipilimumab therapy. Two of the six patients were found to have hypogonadotropic hypogonadism requiring sex hormone replacement, and only one patient had biochemical evidence of growth hormone deficiency. Three of the six patients had evidence suggestive of hypophysitis on brain imaging. Our observations demonstrate six examples of ipilimumab therapy-associated pituitary hormone deficiencies. Ipilimumab use was associated with secondary adrenal insufficiency and secondary hypothyroidism in all patients, but also hypogonadotropic hypogonadism and growth hormone deficiency in a subset. These findings may suggest that ipilimumab enhanced immune therapy selectively target anterior pituitary tissue; particularly corticotrophs and thyrotrophs. Patients receiving ipilimumab therapy should be monitored for anterior pituitary deficiencies

(1) Chambers CA et al., *Annu Rev Immunol* 2001 19 565-594.

(2) Hodi FS et al., *N Engl J Med* 2010 363 711-723.

(3) Dillard T et al., *Pituitary* 2010 13 29-38.

(4) Min L et al., *Eur J Endocrinol.* 2010 Nov 18.

Nothing to Disclose: LM, AV, CB

Pub # P2-423

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Endocrine Influence on Adipose Tissue (1:30 PM-3:30 PM)

Title Questioning the Postprandial Inflammatory Hypothesis -- Absence of Significant Inflammation and No Change in Total or High-Molecular-Weight Adiponectin after a High-Fat Meal in Obesity or Type 2 Diabetes

Author String LK Phillips, JM Peake, X Zhang, IJ Hickman, BE Huang, P Simpson, SH Li, JP Whitehead, JH Martin, JB Prins
University of Queensland, Diamantina Institute, Brisbane, Australia; The Second Affiliated Hospital of Chongqing Medical University, Chongqing, China; CSIRO, Brisbane, Australia; Medical College of Wisconsin, Milwaukee, WI; Mater Medical Research Institute, Brisbane, Australia

Body **Context** : Recent work suggests that macronutrients are pro-inflammatory and promote oxidative stress. Hyperglycaemia and hyperlipidaemia are emerging as important cardiovascular risk factors and thus the postprandial period is a critical area of study. Concentrations of adiponectin, an anti-inflammatory, insulin-sensitising adipokine are reduced in obesity and type 2 diabetes (T2DM). Reports of postprandial regulation of total adiponectin have been mixed, and there is limited information regarding postprandial high molecular weight (HMW) adiponectin.

Objective : To assess the effect of a standardized high fat meal on metabolic parameters, markers of inflammation, indicators of oxidative stress and adiponectin (total and HMW) in: i) lean controls, ii) obese non-diabetic and iii) subjects with T2DM.

Design and Setting : Male subjects: controls (n=10), obese (n=10) and T2DM (n=10) were studied for 6 hours following a high fat meal and water control. Metabolic parameters (glucose, insulin, triglycerides), inflammatory markers (IL6, TNF α , hsCRP, NF[kappa]B expression in peripheral blood mononuclear cells (p65)), indicators of oxidative stress (oxLDL and isoprostanes) and total and HMW adiponectin were measured. Results were analysed using a random coefficient model.

Results : Significant changes in IL6 and hsCRP were seen following both control and meal intervention in all groups. When control data was taken into account, postprandial IL6 decreased in subjects with T2DM but increased in lean subjects, whereas hsCRP decreased in lean and increased in obese subjects. There were no changes in TNF α , p65, oxLDL or protein carbonyl concentrations. No significant postprandial regulation of total or HMW adiponectin was observed in any group. However, following water intervention total adiponectin increased in lean subjects while a decrease was observed in subjects with T2DM.

Conclusions : This study does not support the consistent induction of a significant inflammatory milieu following a high-fat meal. There was no postprandial regulation of adiponectin. A differential total adiponectin response in lean subjects vs. subjects with T2DM following water control was observed, however this study was not designed to assess diurnal regulation of adiponectin and further work in this area would be of interest. This study highlights the imperative for appropriate controls in meal studies and may explain some of the conflicting data evident in this area.

Nothing to Disclose: LKP, JMP, XZ, IJH, BEH, PS, SHL, JPW, JHM, JBP

Pub # P2-424

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Endocrine Influence on Adipose Tissue (1:30 PM-3:30 PM)

Title Age-Dependent Analysis of Leptin and Adiponectin in Dwarf Growth Hormone Receptor Antagonist Mice

Author String E Lubbers, JJ Kopchick, A Jura, V Magon, EO List, DE Berryman
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Body Growth Hormone receptor Antagonist (GHA) mice are dwarf and obese, but unlike other models with decreased growth hormone action, they are not long-lived and do not have improved insulin sensitivity at least at older ages. Levels of several adipokines have been reported in these mice. At 24 weeks of age, both leptin and total adiponectin are increased in the male GHA mice when compared to wild type controls. The data for leptin is somewhat expected as levels tend to directly correlate with fat mass in humans and animals. However, adiponectin is generally reported to negatively correlate with fat mass, yet, in GHA mice, adiponectin is positively correlated with obesity. Adiponectin is a powerful insulin sensitizer, with the high molecular weight (HMW) form possessing the predominate insulin sensitizing bioactivity. Thus, high levels of total adiponectin in GHA mice may be more indicative of its role in glucose homeostasis although HMW levels of adiponectin have not been previously measured in these mice. In addition to the levels of these individual adipokines, the adiponectin to leptin ratio (A/L) has been reported to be better correlated with insulin resistance and atherosclerosis in humans than either leptin or adiponectin alone. Therefore, the purpose of this study was to assess the levels of circulating leptin, total adiponectin, HMW adiponectin and A/L by ELISA in male and female GHA mice throughout life. Circulating leptin levels increased dramatically in GHA mice over lifespan, reaching levels approximately five and eight times higher in females and males, respectively, when compared to wild type controls. In contrast, circulating levels of total and HMW adiponectin were relatively constant throughout life. There were notable gender differences with circulating total adiponectin being higher in females than in males for both genotypes and at all time points. Circulating HMW adiponectin was also increased in female GHA mice in comparison to males at select time points. The A/L was higher in females than males and decreased over life in all groups. By 72 weeks of age, all groups have similar A/L ratios. Collectively, these data confirm previous reports for leptin and adiponectin in males at younger ages and suggest significant gender difference in adipokine expression. Further, as advancing age dramatically altered only leptin, the altered ratio of these adipokines could influence the lifespan of GHA mice.

Sources of Research Support: NIDDK (DK064905); NIA (AG031736); NIA (AG031736); NIDDK (DK075436); NIA (AG19899 and AG031736); NIDDK (DK075436); the State of Ohio's Eminent Scholar Program that includes a gift from Milton and Lawrence Goll, ; a grant from DiAtheGen LLC.; Diabetes Research Initiative at Ohio University; grant from AMVETS.

Nothing to Disclose: EL, JJK, AJ, VM, EOL, DEB

Pub # P2-425

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Endocrine Influence on Adipose Tissue (1:30 PM-3:30 PM)

Title Follicular Fluid Leptin/Adiponectin Ratio and Age Negatively Correlate with Anti-Müllerian Hormone Gene Expression in Human Luteinized Mural Granulosa Cells

Author String ZO Merhi, E Buyuk, D Israel, GM Messerlian, EE Eklund, S Chua, Jr, S Jindal
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Body

Background: Granulosa cells (GC) have receptors (R) for leptin and adiponectin and it has been shown that adiponectin causes an increase in human GC steroidogenesis while leptin inhibits this effect (1,2). Ovarian reserve, reflected by serum anti-Mullerian hormone (AMH) produced by GC, declines with age and obesity (3). We hypothesize that obesity affects AMH/AMH-R gene expression in human luteinized GC through alterations in follicular fluid (FF) leptin and adiponectin that are associated with obesity.

Materials and Methods: 6 early reproductive-aged (<35 years old, BMI= 25.3 ± 2.7 kg/m²; BMI range: 19.1-38.4 kg/m²) and 15 late reproductive-aged ([ge]35 years old, BMI= 26.7 ± 1.6 kg/m²; BMI range: 20.3-40.5 kg/m²) women who underwent controlled ovarian hyperstimulation followed by oocyte retrieval were enrolled. Mural and cumulus GC from small follicles (SF<14 mm) and large follicles (LF[ge]14 mm) were collected separately. The mRNA was isolated, reverse transcribed and AMH/AMH-R gene expression was quantified using RT-PCR. Relative gene expression was calculated using the 2^{-[Delta][Delta]CT} method with GAPDH as reference gene. FF AMH, leptin and adiponectin were measured. Mann-Whitney U test and Spearman's rank correlation were used.

Results: Late reproductive-aged women had significantly lower AMH, but not AMH-R, gene expression in the mural GC of LF (P=0.04) and SF (P=0.01) than early reproductive-aged women. There was no change in cumulus GC AMH gene expression with age. In all participants, FF AMH significantly declined with age (r=-0.6, P=0.01). There was a trend towards lower AMH gene expression in mural GC of SF with increase in BMI (r=-0.49, P=0.1). BMI correlated positively with FF leptin (r= 0.71, P= 0.027) and negatively with FF adiponectin (r= -0.46, P= 0.04). The FF leptin/adiponectin ratio correlated negatively with AMH gene expression in mural GC of SF (r= -0.99, P=0.01).

Conclusion: We report for the first time a negative association between human mural GC AMH gene expression and aging, as well as with FF leptin/adiponectin ratio, a functional indicator of obesity. This suggests that women with altered leptin/adiponectin levels may have impaired AMH production in their mural GC of developing follicles, which adversely affects their ovarian reserve and which is consistent with the poor response and outcomes seen among obese women seeking infertility treatment. The causality of these associations is currently being studied.

1) Adiponectin increases insulin-like growth factor I-induced progesterone and estradiol secretion in human granulosa cells. Chabrolle C, Tosca L, Ramé C, Lecomte P, Roy[grave]re D, Dupont J. *Fertil Steril.* 2009;92:1988-96.

2) Leptin antagonizes the insulin-like growth factor-I augmentation of steroidogenesis in granulosa and theca cells of the human ovary. Agarwal SK, Vogel K, Weitsman SR, Magoffin DA. *J Clin Endocrinol Metab.* 1999;84:1072-6.

3) Association of anti-mullerian hormone levels with obesity in late reproductive-age women. Freeman EW, Gracia CR, Sammel MD, Lin H, Lim LC, Strauss JF 3rd. *Fertil Steril.* 2007;87:101-6.

Sources of Research Support: Ferring Pharmaceutical Grant to SJ; Ferring Pharmaceutical Grant to ZM through New England Fertility Society.

Disclosures: GMM: Researcher, Bechman Coulter Inc. Nothing to Disclose: ZOM, EB, DI, EEE, SC, SJ

Pub # P2-426
Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Endocrine Influence on Adipose Tissue (1:30 PM-3:30 PM)
Title Analysis of the Expression Profile in 3T3-L1 Adipocytes by shRNA-Mediated Silencing of Adipogenin Gene
Author String S-h Song, K-h So, Y Suzuki, Y Kikuchi, A Ardiyanti, K Sato, S-g Roh, K Katoh
Tohoku University, Sendai, Japan

Body Adipocyte differentiation, which is known as adipogenesis, is regulated by a complex array of extracellular signals, intracellular mediators, and transcription factors. We isolated a new regulating gene called adipogenin gene, which was highly expressed in subcutaneous and visceral adipose tissues of mice fed with high-fat diet compared with control-diet group. This gene was also up-regulated during 3T3-L1 preadipocyte differentiation and development [1]. However, the role and mechanism of adipogenin on fat accumulation and adipocyte differentiation remain largely unknown.

Our present study aimed to analyze the gene expression profiles on adipocyte differentiation of adipogenin knock-down 3T3-L1 cells. Knock-down of adipogenin caused a lower fat accumulation in 3T3-L1 cells compared with control, negative, and positive control cells. Moreover, RT-PCR analysis showed that the expression levels of adipogenic differentiation genes, i.e. SREBP, C/EBP, PPAR γ -2, and glucose uptake gene i.e. GLUT4, were decreased by knockdown of adipogenin. cDNA microarray was carried out to identify adipogenin-regulated target gene by comparing the expression profiles of 3T3-L1-negative controls and 3T3-L1-sh-adipogenin cells. There were 718 and 508 genes up-regulated and down-regulated, respectively, by adipogenin found with over two fold changes. Pathway analysis showed that an increased expression level of adipogenin gene up regulated adipoQ and GLUT4 genes expression levels, fatty acid oxidation, and uptake, transport, and metabolism of glucose. These data suggest that adipogenin acts as adipogenic and fat accumulation factor in adipocytes. Expression analysis showed that genes down-regulated upon adipogenin knock-down in 3T3-L1 cells were significantly enriched to PPAR γ and transcription factors, i.e. SP1, JUN, STAT3, and PPAR α . On the other hand, genes down-regulated upon adipogenin knock-down in 3T3-L1 cells were significantly enriched to Col18a1, CXCL12, and AGRN. Most of the transcription factors shown in our results were connected with inflammatory genes, such as IL-18 and chemokines. These results showed that adipogenin is one of the important factors involved in fat accumulation, adipocyte differentiation, and other biological function.

[1] Hong YH, Hishikawa D, Miyahara H, Tsuzuki H, Nishimura Y, Gotoh C, Choi KC, Hokari Y, Takagi Y, Lee HG, Cho KK, Roh SG, Sasaki S. (2005). "Up-regulation of adipogenin, an adipocyte plasma transmembrane protein, during adipogenesis." *Mol Cell Biochem* 276(1-2):133-141.

Nothing to Disclose: S-HS, K-HS, YS, YK, AA, KS, S-GR, KK

Pub # P2-427

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Endocrine Influence on Adipose Tissue (1:30 PM-3:30 PM)

Title Vitamin D May Modulate Adiponectin Levels in Women

Author String KS Gravenstein, R Ramachandran, K Farhang, JM Egan, L Ferrucci, CW Chia
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Body Background: Low adiponectin levels are a risk factor for insulin resistance and type 2 diabetes, and therefore, increasing adiponectin levels may reduce the risk of these conditions. Emerging evidence suggests that vitamin D deficiency negatively affects glucose metabolism. Vitamin D deficiency has been linked to insulin resistance, metabolic syndrome, visceral adiposity and risk of diabetes. We hypothesize that the effect of vitamin D on glucose homeostasis may be due to modulation of adiponectin secretion from adipocytes. This hypothesis is supported by the presence of vitamin D receptors in adipocytes.
Methods: This analysis used data from 348 men and 374 women in the Baltimore Longitudinal Study of Aging who were not on glucose-lowering medications. Using multiple linear regression, we tested the association between total plasma adiponectin and serum 25-hydroxyvitamin D (25(OH)D), adjusting for multiple confounders including insulin resistance assessed by the homeostatic model (HOMA2-IR). Adiponectin and HOMA2-IR were log-transformed for analyses. We found a significant interaction between sex and age ($P < 0.01$); therefore we stratified analyses by sex. Data are expressed as mean \pm SD and standardized regression coefficients (β).
Results: Men and women differed by age (70 \pm 13 vs. 67 \pm 13 yrs), adiponectin (12.7 \pm 10.4 vs. 17.2 \pm 11.7 [μ g/mL), 25(OH)D (32.3 \pm 10.7 vs. 35.5 \pm 16.0 ng/mL), and fasting glucose (92 \pm 11 vs. 89 \pm 10 mg/dL), $P < 0.05$ for all. Men and women had similar BMI (27.4 \pm 4.1 vs. 26.8 \pm 5.2 kg/m²), fasting insulin (9.1 \pm 6.7 vs. 8.7 \pm 5.9 [μ U/mL), and HOMA2-IR (1.2 \pm 0.9 vs. 1.1 \pm 0.8). In multiple linear regression analysis, adiponectin was independently associated with 25(OH)D in women ($\beta = 0.14$; $p < 0.05$) but not in men ($\beta = -0.005$; $p = 0.9$).
Conclusions: Vitamin D levels may influence adiponectin levels, at least in women. Further research is needed to determine whether this effect of 25(OH)D significantly influences glucose metabolism and whether 25(OH)D supplementation may reduce insulin resistance and prevent diabetes.

Sources of Research Support: Intramural Research Program of the NIH, National Institute on Aging.

Nothing to Disclose: KSG, RR, KF, JME, LF, CWC

Pub # P2-428

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Endocrine Influence on Adipose Tissue (1:30 PM-3:30 PM)

Title 25-Hydroxyvitamin D Is an Independent Positive Predictor of Circulating Adiponectin in Two Large Cohorts

Author String A Vaidya, JS Williams, JP Forman
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Body Background: The relative deficiencies of 25-hydroxyvitamin D (25[OH]D) and adiponectin have both been associated with obesity and cardiovascular disease. Prior cross-sectional studies have suggested that 25(OH)D concentrations are positively associated with circulating adiponectin, and that this relationship may strengthen with increasing body-mass index (BMI). However, these prior studies were small in size and did not account for many known confounders of adiponectin levels.
Objective: To evaluate the independent association between 25(OH)D and adiponectin in two large populations, and to determine whether BMI modifies this relationship.
Methods: Cross-sectional analyses were performed on 1206 women from the Nurses' Health Study I and 439 men from the Health Professionals Follow-Up Study. Multivariable linear regression was used to analyze whether 25(OH)D was associated with adiponectin independent of age, race, BMI, physical activity, diabetes status, hypertension status, anti-hypertensive drug use, alcohol intake, a prudent dietary pattern, and dietary intakes of sodium, calcium, and potassium. Analyses were also adjusted for case-control status, and menopausal status in women. Effect modification by BMI was examined by creating interaction terms between vitamin D and BMI.
Results: Women had higher circulating adiponectin (17.9 [7.7] vs. 10.0 [8.1] [μ g/mL), but lower 25(OH)D concentrations (24.4 [9.4] vs. 28.7 [9.9] ng/mL), when compared to men. 25(OH)D concentrations were positively associated with adiponectin (women: $\beta=0.12$, $P<0.001$; men: $\beta=0.08$, $P<0.05$), even after multivariable adjustments (women: $\beta=0.06$, $P<0.001$; men: $\beta=0.07$, $P<0.05$). When evaluating the standardized associations with adiponectin, BMI expectedly displayed the largest effect estimates (women: $ST\beta=-0.24$, $P<0.001$; men: $ST\beta=-0.16$, $P<0.001$); however, 25(OH)D demonstrated the second largest standardized effect estimate in women ($ST\beta=0.08$) and the third largest in men ($ST\beta=0.08$). BMI did not modify the relationship between 25(OH)D and adiponectin.
Conclusions: Higher 25(OH)D concentrations were independently associated with higher adiponectin concentrations in large populations of men and women. Since 25(OH)D and adiponectin deficiency are associated with unfavorable cardio-metabolic profiles, future studies to assess the effect of vitamin D supplementation on adiponectin levels are warranted.

Nothing to Disclose: AV, JSW, JPF

Pub # P2-429

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Endocrine Influence on Adipose Tissue (1:30 PM-3:30 PM)

Title Selective Leptin Resistance in Brain but Not in Adipose-Tissue Macrophages Following High-Fat Feeding

Author String D Athonvarangku, K Zhang, W Li, P Kishore, M Hawkins
Albert Einstein College of Medicine, Bronx, NY

Body Although leptin resistance in obese individuals has precluded use of this adipokine as an 'anti-obesity' therapeutic, recent evidence for selective leptin resistance suggests that leptin still fuels hypertension and other complications of obesity despite loss of its beneficial metabolic effects (1). We previously showed that central leptin administration rapidly induced adipose tissue macrophage (ATM) activation and adipose tissue inflammation in normal rats (2), consistent with leptin's known effects on immunity. Given the phenomenon of selective resistance, we hypothesized that leptin's stimulatory effects on ATMs would persist with high-fat feeding. We compared the effects of intracerebroventricular leptin 0.25 [μ]g/h (n=6) vs. vehicle (n=6) in normal male rats following 5 days of high fat feeding. While this duration of high fat feeding induced weight gain and abolished the suppressive effects of leptin on food intake, persistent stimulatory effects of leptin on ATMs were evidenced by increased cytokine gene expression by real-time PCR (2.5-fold in TNF- α , 2.8-fold in IL-6 and 1.9-fold in PAI-1, all p <0.05) and cytokine production by immunofluorescence (1.6-fold increase in adipose iNOS production in leptin-treated rats in association with an ATM-specific marker). Additionally, we believe that central administration of leptin has measurable effects on the ability of adipocyte-derived factors to impact macrophages. Relative to adipocyte medium from saline-treated rats, adipocyte medium from leptin-treated rats induced greater activation of RAW264.7 macrophages, with increased expression of TNF- α (49% increase) and iNOS (175% increase), though no significant rise in IL-6 (9% increase). Of note, the cultured macrophage cells also manifested a more 'reactive' appearance when exposed to adipocyte medium from leptin-treated rats. Overall our results show that, despite resistance to leptin's effects on food intake and energy balance after 5 days of high fat feeding, adipose tissue macrophages remain sensitive to leptin's stimulatory effects on their expression of key inflammatory cytokines. Further understanding of selective leptin resistance may help to elucidate the pathogenesis of various metabolic complications of obesity, including adipose tissue inflammation.

(1) Kaiyala KJ et al., Diabetes 2010; 59(7):1657

(2) Zhang et al., Diabetes 2008; 57(suppl 1):281A

Sources of Research Support: DK048321.

Nothing to Disclose: DA, KZ, WL, PK, MH

Pub # P2-430
Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Endocrine Influence on Adipose Tissue (1:30 PM-3:30 PM)
Title CTRP-3 Represents a Novel and Endogenous LPS Antagonist of the Adipose Tissue
Author String A Kopp, F Hanses, W Falk, A Schaeffler
University of Regensburg, Regensburg, Germany

Body

Introduction

Proteins secreted by adipocytes (adipokines) play an important role in the pathophysiology of type 2 diabetes mellitus and the associated chronic and low-grade state of inflammation. It was the aim to characterize the antiinflammatory potential of the new adipocytokine, C1q/TNF-related protein-3 (CTRP-3), which shows structural homologies to the pleiotropic adipocytokine adiponectin.

Methods

mRNA and protein expression of CTRP-3 was analyzed by RT-PCR and Western blot. Recombinant CTRP-3 and small interfering RNA-based strategies were used to investigate the effect of CTRP-3 on toll-like receptor (TLR) ligand, lipopolysaccharide (LPS)-, and lauric acid-induced chemokine release in monocytes and adipocytes. A designed TLR4/myeloid differentiation protein-2 fusion molecule shown to bind LPS was used to prove the ability of CTRP-3 to act as endogenous LPS antagonist. The effectiveness of CTRP-3 to block LPS-induced proinflammatory response was tested in a murine model of LPS-induced SIRS (severe inflammatory response syndrome).

Results

CTRP-3 is synthesized in monocytes and adipocytes. The recombinant protein dose-dependently inhibits the release of chemokines in monocytes and adipocytes that were induced by lauric acid, LPS, and other TLR ligands in vitro and ex vivo. CTRP-3 inhibits monocyte chemoattractant protein-1 release in adipocytes, whereas small interfering RNA-mediated knockdown of CTRP-3 up-regulates monocyte chemoattractant protein-1 release, reduces lipid droplet size, and decreases intracellular triglyceride concentration in adipocytes, causing a dedifferentiation into a more proinflammatory and immature phenotype. By using a designed TLR4/MD-2 fusion molecule, it is shown by different techniques that CTRP-3 specifically and effectively inhibits the binding of LPS to its receptor, TLR4/MD-2. In LPS-injected mice, CTRP-3 given intraperitoneally (1 µg, 10 µg) significantly reduced LPS-induced serum concentrations of cytokines and chemokines such as IL-6, IL-8, TNF, and MCP-1. Moreover, inguinal adipose tissue expression of LPS-induced IL-6 and IL-8 mRNA was reduced significantly by CTRP-3.

Conclusion

CTRP-3 inhibits basic and common proinflammatory pathways involved in obesity and type 2 diabetes mellitus (adipo-inflammation) by acting as an endogenous LPS antagonist of the adipose tissue.

Sources of Research Support: German Research Association (DFG).

Nothing to Disclose: AK, FH, WF, AS

Pub # P2-431

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Endocrine Influence on Adipose Tissue (1:30 PM-3:30 PM)

Title The Potential Role of Neuropeptide Y Expression in the Dorsomedial Hypothalamus in Thermoregulation during Lactation and Diet-Induced Obesity

Author String SJ Draper, A Loche, CJ Madden, S Smith, SF Morrison, KL Grove
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Body The dorsomedial hypothalamus (DMH) has emerged as one of the key thermoregulatory sites in the hypothalamus, as well as playing a role in the regulation of food intake and metabolism. Anatomical and functional studies have shown that the neurons located in dorsal aspect of the DMH stimulate brown adipose tissue (BAT) thermogenesis via the rostral medullary raphe pallidus (rRPa). However, the phenotype of the DMH neurons projecting to the rRPa is unknown. Interestingly, the DMH contains neurons that express neuropeptide Y (NPY) during hyperphagic/obese conditions in rodents and the temporal expression of NPY in the DMH is closely associated with decreased BAT thermogenesis and increased food intake in lactating rats. The goal of the present study is to identify the anatomical connections between DMH-NPY neurons and brain sites that regulate thermogenesis in lactating rats and diet induced obese mice (DIO). The anterograde tracer, biotinylated dextran amine (BDA, 60nl), was stereotaxically injected into the DMH of lactating rats and DIO mice. After allowing BDA transport for 5-7days, the brains were collected for immunohistochemical visualization of BDA and NPY fibers. The BDA fiber projections were comparable to the previously reported DMH projections using other neuronal tracers. In the rRPa and the paraventricular nucleus (PVH) area, confocal microscopy revealed a small number of fibers double-labeled for BDA and NPY, indicative of direct projections from the DMH-NPY neurons. These data provide an anatomical substrate through which DMH-NPY neurons could regulate BAT thermogenesis during lactation or hyperphagia. The PVH also modulates food intake and energy expenditure via endocrine and sympathetic pathways. Further studies will characterize the cell types in the target areas and also test the functional connection between DMH-NPY expression and BAT thermogenesis.

Nothing to Disclose: SJD, AL, CJM, SS, SFM, KLG

Pub # P2-432

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Endocrine Influence on Adipose Tissue (1:30 PM-3:30 PM)

Title Leptin Antagonists Induce Weight Gain and Insulin Resistance: Early Effects on Metabolism and Activity

Author String A Gertler, WA Banks, G Solomon, KM Lynch, T Wolden-Hanson
The Hebrew University, Rehovot, Israel; Veterans Affairs Health Care Puget Sound Health Care System, Seattle, WA

Body Pegylated mouse leptin antagonists - PEG-MLA (L39A/D40A/F41A) and superactive mouse leptin antagonists - PEG-SMLA (D23L/L39A/D40A/F41A) are potent orexigenic stimulators. We showed that PEG MLA induces peripheral leptin resistance by blocking its ability to cross the BBB. Here, we found that IP injections of PEG-MLA (20 mg/kg/day) or PEG-SMLA (5 mg/kg/day) for 2-3 weeks to male mice increased weight gain by 25 and 45% due to fat accumulation and could be reversed by ceasing the injections. Male mice (n=16) injected with PEG-MLA (20 mg/kg/day) for 21 days gradually developed insulin resistance and the difference in insulin level and HOMA compared to the controls was significant ($p<0.05$). In a short term metabolic experiment, male mice, acclimatized to a metabolic chamber received morning, SC injections of either PEG-SMLA (5 mg/kg/day) or vehicle. These injections were repeated 24 h later and the mice studied in a metabolic chamber for 48 h, beginning with the first injection and ending 24 h after the second injection. By the end of the 2nd 24 h period, mice that had received SMLA weighed 3 g more than those receiving vehicle ($p<0.05$), had an increase in RQ consistent with conservation of fat mass ($p<0.05$), and a reduction in activity ($p<0.05$). There were no differences in oxygen consumption and total energy expenditure. These results show that blockade of leptin with leptin antagonists induced weight gain and protected fat mass and suggest they may be useful in the treatment of anorexia and in developing models of insulin resistance.

Nothing to Disclose: AG, WAB, GS, KML, TW-H

Pub # P2-433

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Endocrine Influence on Adipose Tissue (1:30 PM-3:30 PM)

Title Compartmental Analysis of Leptin in Tissues of Mice

Author String R Hart, R Dobos, J McFarlane
University of New England, Armidale, Australia; Industry & Investment NSW, Armidale, Australia

Body Leptin is the product of the ob gene and was originally thought to be made by adipose tissue exclusively, with the brain the primary target as part of the appetite and energy regulation feedback loop. Further research has shown leptin to be made in other tissues and involved in many other systems. There have been relatively few pharmacokinetic studies of leptin and although it has been demonstrated that many different tissues contain leptin receptors these studies have mostly developed 2 pool models and no study has attempted to develop a more extensive compartmental model.

The tissue distribution of radiolabelled leptin over time was investigated in adult female Swiss outbred mice using the modeling software WinSAAM to develop a compartmental model. Mice were injected with radiolabelled leptin into the tail vein and then sacrificed at 5, 15, 30 and 60 minutes after injection. Blood and tissues were collected, weighed and the radioactivity determined.

The plasma half-life of leptin in the initial rapid clearance phase was 6.3 minutes while in the slower clearance phase it was 178.4 minutes similar to that reported in the literature. The majority of leptin binding was found to be in the gastrointestinal tract particularly the stomach and small intestines, kidneys, liver and skin, lesser amounts were found in many other tissues including the brain. We developed an initial 6 pool compartmental model of leptin pharmacokinetics using the sites where the majority of leptin bound and the brain. The fitted model predicts two way transfers into and out of plasma for the brain, liver and skin and a one way transfer out of plasma into the GI tract and the kidney. The key findings from this analysis are that a large portion of leptin originated in the brain, followed by the liver and skin. Disposal of leptin was primarily from the kidney with some via the GI tract.

This model integrates information on leptin distribution in mice to enable the testing of hypotheses on leptin pharmacokinetics. Initial results indicate the model while a significant step forward requires refinement in the area of adipose tissue as currently skin is used as a proxy. Future development of the model would require integration of data from experiments where leptin has been injected into the brain and incorporation of other tissues with significant leptin binding.

Nothing to Disclose: RH, RD, JM

Pub # P2-434

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Endocrine Influence on Adipose Tissue (1:30 PM-3:30 PM)

Title Treatment with a Long-Acting Peptide YY Decreases Food Intake in Mice without Eliciting Common Side Effects

Author String A Qadri, K Higgins, K Coughlan, AM Richard, Q Huang, J Jones, V Clerin, M Perreault, A Lake
Pfizer, Cambridge, MA

Body Peptide YY (PYY) is a 36-amino acid gut hormone belonging to the pancreatic polypeptide family. Cleavage of the first two amino acids results in PYY3-36 which is selective for the NPY Y2 receptor (Y2R). Peripheral administration of PYY3-36 decreases food intake and body weight in humans, non-human primates and rodents; thus a long-acting agonist at Y2R has the potential to be an effective anti-obesity therapeutic. Addition of a PEG moiety to the PYY3-36 peptide (PEG-PYY) greatly enhanced in vivo peptide stability while maintaining potency and selectivity for Y2R. As a result, a single intraperitoneal injection of PEG-PYY (10-100upk) elicited a significant decrease in food intake out to 6h post-dose in lean mice, while native PYY (400upk) was only efficacious out to 30 min post-dose. To further evaluate behavioral and physiological alterations elicited by peripheral PEG-PYY administration, locomotor activity, blood pressure, heart rate and malaise were evaluated. First, while peripheral administration of PEG-PYY (100upk) decreased food intake, this change did not elicit malaise (assessed by a conditioned taste aversion paradigm, which is a correlate to human nausea) when compared to a higher dose of PEG-PYY (400upk) or Lithium Chloride (120mpk). Next, we examined the effect of escalating doses of PEG-PYY (0.1, 0.3, 1.0, 3.0mpk), on locomoter activity, blood pressure and heart rate. At 0.1 and 0.3 mpk, which decreases food intake, little to no alterations in cardiac parameters were observed. At higher doses (1.0 and 3.0mpk), treatment with PEG-PYY resulted in a dose-dependent increase of blood pressure and heart rate, which correlated with increased activity during the light cycle. In the dark cycle, the highest dose of PEG-PYY (3mpk) produced a robust decrease in locomotor activity and lowered blood pressure, which is consistent with a decrease in food seeking behavior. These data suggest that a modified, long-acting Y2R agonist may be useful for the treatment of obesity without eliciting the major side effects (cardiac and nausea) observed with the unmodified peptide.

Nothing to Disclose: AQ, KH, KC, AMR, QH, JJ, VC, MP, AL

Pub # P2-435

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Endocrine Influence on Adipose Tissue (1:30 PM-3:30 PM)

Title Leptin Administered in Physiologic or Pharmacologic Doses Does Not Regulate Circulating Angiogenesis Factors in Humans

Author String KN Arons, CG Fiorenza, JP Chamberland, CS Mantzoros
Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA; Harvard School of Public Health, Boston, MA; Boston VA Healthcare System, Harvard Medical School, Boston, MA

Body
Introduction: Leptin has been shown to regulate angiogenesis in animal and *in vitro* studies by upregulating the expression of several pro-angiogenic factors, but its role in regulating angiogenesis has never been studied in humans. Our aim was to evaluate whether leptin has a role in regulating circulating angiogenic factors in humans.
Methods: We conducted 2 studies. First, a short-term, interventional study of 15 healthy, normoleptinemic men and women, to whom metreleptin was administered in escalating doses (physiologic, 0.1 mg/kg, and pharmacologic, 0.3 mg/kg) on two admissions separated by 1-12 weeks. Serum was collected at 0, 6, 12, and 24 hrs post-metreleptin to evaluate its acute effects on angiogenesis. Second, a prospective, double-blinded RCT of 20 women with hypothalamic amenorrhea and hypoleptinemia (leptin levels < 5 ng/ml) that were randomized in a 1:1 fashion to receive either metreleptin (0.08-0.12 mg/kg qd) or placebo for 32 weeks. Serum was collected at 0, 8, 20, and 32 wks after randomization to evaluate the long-term effects of metreleptin in replacement doses in hypoleptinemic amenorrheic subjects. Main outcome measures included circulating levels of angiogenin, angiopoietin-1, PDGF-AA, MMP-8 and 9, EGF, and VEGF. Statistical Analysis: variables were examined for normality, and appropriate transformations were applied where appropriate. Comparisons were made with repeated measurements analysis of variance in the short term study and Hierarchical Linear Models in the long term study.
Results: Although leptin levels significantly increased following both acute and chronic metreleptin administration, circulating concentrations of angiogenesis markers did not change significantly.
Conclusions: This is the first study that examined the effect of leptin administration in angiogenesis in humans. Metreleptin administration does not regulate circulating angiogenesis factors in humans, suggesting that leptin does not regulate angiogenesis in humans.

Sources of Research Support: National Institutes of Health - National Center for Research Resources grant M01-RR-01032 (Harvard Clinical and Translational Science Center) and grant number UL1 RR025758. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Center for Research Resources or the National Institutes of Health. Funding was also received from the National Institute of Diabetes and Digestive and Kidney Diseases grants 58785, 79929 and 81913, and AG032030. Amylin Pharmaceuticals, Inc. supplied metreleptin for this study but had no role in the study design; conduct of the study; collection, management, analysis, and interpretation of the data; or the preparation, review, or approval of the manuscript.

Nothing to Disclose: KNA, CGF, JPC, CSM

Pub # P2-436

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Endocrine Influence on Adipose Tissue (1:30 PM-3:30 PM)

Title Ghrelin Simultaneously Stimulates Growth Hormone Release and Food Intake in an Undisturbed Pig Model of Obesity

Author String K Raun, P von Voss
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Body Ghrelin is a gastric peptide that binds to and activates growth hormone releasing (GHS) receptors, which primarily are located in the arcuate nucleus in the hypothalamus an area important for growth hormone (GH) release and appetite regulation. Ghrelin has been shown to have a positive effect on GH release and appetite regulation in several species inclusive human. GH is released in a very pulsatile manner, demanding profiles consisting of frequently obtained samples in order to make reliable investigations of effect on GH secretion. The purpose of this study was to simultaneously investigate the GH releasing effect and appetite stimulatory effect of porcine ghrelin in undisturbed ad libitum fed obese minipigs. Furthermore to investigate effect on glucose and insulin levels. Four adult moderate obese female G[ouml]ttingen minipigs (64.3±3 kg) were fixed with jugular catheters under anesthesia. After recovery the catheters were attached to an automatic blood sampling/infusion system (AccuSampler[reg]) in a manner, that allowed the animals to move unstressed and freely around in their pens with ad libitum access to food throughout the experiment. Blood samples were drawn automatically from the accusampler every 15 min for 24 hours (h) (basal period) and every 15 min for 24 h after a single administration of porcine ghrelin given iv in a bolus dose of 80 [mu]g/kg. Plasma was analyzed for porcine GH. Food intake was automatically monitored every 15 min throughout the experiment by an online system developed by Ellegaard (MPIGWin). The iv ghrelin injection resulted in a rapid and significant GH peak with a cmax of 22.5±5.0 ng/ml compared to 1.6±1.1 ng/ml (p<0.01) for the corresponding time during basal period. This led to a significant increase in 4 h GH AUC (ng/ml * min) of 1603± 494 compared to 425±299 (p<0.01) for the corresponding basal period. The increase in GH levels lasted for 24 h (p<0.05) in the ghrelin treated pigs compared to the 24 h in the basal period in the same pigs (p<0.05). Also a significant increase in food intake was seen during the first 4 h (715 ±133 g) after ghrelin treatment compared to the first 4 h in the basal period (99 ± 97 g) (p<0.05). No difference was found in 24 h accumulated food intake. Both glucose and insulin increased during the first 4 h after ghrelin (p<0.01) probably due to increased food intake. In conclusion porcine ghrelin acutely stimulates GH release as well as stimulates appetite in an obese ad libitum fed minipig model.

Nothing to Disclose: KR, PvV

Pub # P2-437

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Endocrine Influence on Adipose Tissue (1:30 PM-3:30 PM)

Title Long-Term Metabolic Consequences of Neonatal Exendin-4

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Body Exendin-4 (Ex-4) is a treatment for adult human type 2 diabetes. In rats, neonatal Ex-4 prevents obesity and diabetes in a model of intrauterine growth retardation (IUGR) in part by inducing epigenetic modifications of beta cell genes to normalize beta cell mass and vascularity (1,2,3). Administration of Exendin-4 during this critical window of development also induces a long-term reduction in body weight. Using a similar neonatal paradigm in mice, we examined the short- and long-term effects of neonatal Exendin-4 treatment on body weight using a diet-induced obesity model. Neonatal Ex-4 attenuated weight gain in male C57BL6 mice on a low fat diet (LFD) by 14% by postnatal day 28 ($p < 0.05$). Whereas diminished weight gain in females occurs more dramatically on a high fat diet (HFD), with a maximum decrease of 19% beginning at 6 months of age. Adult HFD-fed females treated with neonatal Ex-4 exhibited a 3% increase in energy expenditure associated with a 26% increase in horizontal beam counts, a 13% decrease in perigonadal fat mass and a 52% decrease in expression of PPAR γ 2 transcript in perigonadal adipose tissue (all $p < 0.05$). There was no change in total food intake or fluid intake, when measured over a 24 h period. These findings suggest that a limited early postnatal treatment with Ex-4 permanently alters adipose development and/or lipid metabolism with a long-term impact on body weight regulation. This phenotype may also involve long-term effects on the central nervous system impacting energy expenditure and locomotor activity.

- (1) Stoffers, D.A. et al., Diabetes 2003; 52:734-740
- (2) Ham, J.N. et al., Pediatr Res 2009; 66(1):42-6
- (3) Park, J.H. et al., J Clin Invest 2008; 118:2316-2324

Sources of Research Support: NIH Grant F32DK085939 awarded to DAB; NIH Grant RO1 DK062965-05 awarded to DAS.

Nothing to Disclose: DAB, APMS, DG, RS, RA, DAS

Pub # P2-438

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Endocrine Influence on Adipose Tissue (1:30 PM-3:30 PM)

Title Polymorphisms of Genes *TAP1* and *APOE* as Risk Factors for Cardiovascular Disease in Turner Syndrome Patients

Author String KC Oliveira, B Bianco, AD Guedes, BB Galera, MF Galera, ITdN Verreschi, MVN Lipay
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Body **BACKGROUND/AIM:** The main cause of mortality in Turner syndrome (TS) patients are cardiovascular diseases (CVD). The arterial hypertension (AH), a condition often associated with TS, with a prevalence ranging between 20% and 50%, represents one of the most important risk factors for CVD. The A637G polymorphism in *TAP1* gene (**transporter 1, ATP-binding cassette**), is associated with the pathophysiology of hypertension, as therefore generates endothelial dysfunction, through the erroneous processing of peptides and amino acids. It is well known the relationship of deleterious changes in lipid profile with CVD. However, the frequency of such patterns, usually associated with cardiovascular risk in TS has not yet been established. The gene for apolipoprotein E (*ApoE*), presents three polymorphic alleles (E2, E3 and E4). The E2 allele is considered a protective allele while the E4 allele is associated with risk of CVD. Thus, the aim of this study is to evaluate the frequency of polymorphisms of these genes in women with TS and associate them to such conditions present in this syndrome.

METHODS OF STUDY: We analyzed 146 patients with TS and 372 individuals, healthy with no familial history of CVD as controls. *TAP1* polymorphisms were investigated by PCR-RFLP and ApoE polymorphisms by qPCR. The findings were statistically analyzed by chi-square test and p values [le] 0.05 were considered significant.

RESULTS: The frequencies of genotypes and alleles of the A637G polymorphism of *TAP1* gene and of the alleles of *APOE* gene were similar between patients and controls.

CONCLUSION: There were no significant associations between *TAP1* gene polymorphisms and isoforms of APOE gene in TS patients, which could explain the higher incidence of hypertension and dyslipidemia in this syndrome.

Sources of Research Support: Grants No. 2008/03597-0 from FAPESP.

Nothing to Disclose: KCO, BB, ADG, BBG, MFG, ITdNV, MVNL

Pub # P2-439

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Endocrine Influence on Adipose Tissue (1:30 PM-3:30 PM)

Title Genetic Evidence for Reducing Obesity by Suppressing Klotho Functions

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Body Klotho is a multifunctional factor involved in numerous biological activities, including mineral ion metabolism, and insulin signaling. Recent studies have identified klotho as a target gene for peroxisome proliferator-activated receptor- γ (PPAR-g), a master regulator of adipocyte differentiation, and found it to be an adipogenesis-promoting factor. In a similar line of observation, eliminating klotho function from mice resulted in a lean mouse with almost no detectable fat tissue in their body [1]. In contrast to the *klotho* knockout mice, leptin-deficient (*ob/ob*) mice are severely obese, due to excessive fat accumulation in their body. To study the *in vivo* role of klotho in obesity, we generated and characterized *ob/ob* mice lacking klotho activity [*ob/ob-klotho* double knockout (DKO) mice]. The *ob/ob* mice started to get bigger from 3 weeks onwards and gained almost three times more weight than their wild-type counterparts. The generated *ob/ob-klotho* DKO mice were not only viable throughout their adulthood, but also showed markedly reduced fat tissue accumulation in their body, compared to their *ob/ob* littermates. The *ob/ob-klotho* DKO mice had significantly less retroperitoneal, mesenteric and epididymal fat accumulation, compared to their *ob/ob* counterparts. In accord with the excessive fat tissue distribution, the fatty liver was also consistently observed in the *ob/ob* mice, and was eliminated in the *ob/ob-klotho* DKO mice. Such structural improvement in the liver was also evident from markedly reduced blood glucose levels in *ob/ob-klotho* DKO mice, compared to their *ob/ob* counterparts. The results of our genetic manipulation studies provide *in vivo* evidence for a role of klotho in obesity, and offer a novel therapeutic target to manipulate obesity and associated complications.

(1) Nakatani et. al., FASEB J. 2009; 23:433-41.

Nothing to Disclose: MO, SK, JA, KT, AA, MSR

Pub # P2-440

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Sex & Adipose Tissue Distribution (1:30 PM-3:30 PM)

Title Impact of Estradiol, ER-Subtype-Specific Agonists and Genistein on Energy Homeostasis in a Rat Model of Nutrition-Induced Obesity

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Body
Introduction: The prevalence of obesity, a major risk for chronic diseases such as type 2 diabetes, insulin resistance, dyslipidemia, cardiovascular disease, and certain forms of cancer, is dramatically increasing. High-calorie nutrition and a lack of physical activity are the main reasons for this global epidemic (Guh et al. 2009, Newbold et al. 2009). Estrogen receptors (ER) are recognized to be involved in many processes related to the control of energy homeostasis (Ropero et al. 2008). Therefore it is of interest to determine to what extent the both distinct ER subtypes ERalpha and ERbeta are involved in the underlying molecular mechanisms.
Aim and Methods: The aim of this study was to investigate the impact of 17 β -estradiol (E2), the ER subtype specific agonists 16 α -LE2 (Alpha) and 8 β -VE2 (Beta), and the phytoestrogen Genistein (Gen) on energy homeostasis in a rat model of nutrition induced obesity. For this purpose, juvenile female Wistar rats were ovariectomized (OVX) or sham-operated (SHAM) and fed with low-fat (LF) and high-fat diet (HF) respectively. A subset of OVX HF rats received E2, Alpha or Beta via ALZET[reg] osmotic mini pumps or genistein via Gen-enriched food. After 10 weeks of treatment, body weight, visceral body fat, and serum levels of leptin and blood lipids were investigated.
Results: The body weight measured in the SHAM-operated groups was significantly lower than in the untreated OVX rats, although we did not find any significant differences in energy intake. Treatment of OVX rats on HF diet with E2 or Alpha could antagonize the OVX induced body weight gain, whereas the application of Beta or Gen had no effect. In terms of adipose tissue, SHAM, E2 and Alpha treated animals, but also Beta treated animals revealed significantly lower visceral fat mass, adipocyte size, and serum leptin level compared to the untreated OVX HF animals. On both diets ovariectomy resulted in significantly higher serum levels of total cholesterol (TC) and the subunits HDL, LDL, and VLDL. Treatment with E2 and Alpha significantly decreased TC and VLDL, whereas the application of Beta or Gen showed no significant impact.
Conclusions: Our data reveal that the body weight as well as the TC and VLDL in the serum are positively affected by E2 via ERalpha. The positive effects of E2 on adipose tissue appear to be mediated via ERalpha and ERbeta. The administration of Gen seems to have no beneficial influence on the investigated parameters in adipose tissue and blood.

Nothing to Disclose: CW, TH, K-HF, PD

Pub # P2-441

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Sex & Adipose Tissue Distribution (1:30 PM-3:30 PM)

Title Age of Vaginal Opening as a Predictor of Leptin Sensitivity in Rats

Author String ML Sleeth, EL Thompson, KE Beale, JE Baxter, SR Bloom, KG Murphy
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Body Age of pubertal onset is influenced by nutritional status. Leptin is an adipocyte-derived cytokine critical to the integration of energy homeostasis and reproduction, putatively acting as a permissive signal for pubertal onset. It is known that leptin resistance is associated with diet induced obesity (DIO). However, it is unknown whether inherent differences in leptin sensitivity influence the age of pubertal onset. If age of pubertal onset is associated with leptin sensitivity, age of pubertal onset may also predict susceptibility to DIO. We hypothesised that leptin sensitivity in female Sprague Dawley rats would correlate with age of vaginal opening (VO), a marker of pubertal onset. Rats were given an intra-peritoneal injection of vehicle or leptin (3mg/kg) at the onset of the dark phase in a cross-over design. Leptin sensitivity was assessed by measuring food intake following leptin vs. vehicle at 4 and 24 hrs post-injection. Leptin sensitivity tests were performed pre-pubertally, peri-pubertally and 4 weeks post-pubertally. Rats were retrospectively classified into early (post-delivery day 33-36) or late (post-delivery day 41-44) VO categories. Following VO, standard chow diet was replaced with a high-fat diet (32% energy from fat) for 4 weeks. Body weight was monitored daily to determine whether age of VO (pubertal onset) predicted susceptibility to DIO. Following 4 weeks of post-pubertal high-fat feeding, females in the early VO category were significantly more sensitive to the anorexigenic effects of exogenous leptin than those in the late VO category. However, there was no significant difference detected in leptin sensitivity between rats which underwent early VO and those that underwent late VO in either the pre-pubertal or peri-pubertal period when fed on standard chow. There was no significant difference in final body weight or food intake between the early and late VO groups. These data suggest that early VO (pubertal onset) in female Sprague Dawley rats may be associated with improved leptin sensitivity after 4 weeks of high fat feeding. However, these data suggest that age of pubertal onset does not predict acute leptin sensitivity in either the pre-pubertal or post-pubertal period.

Sources of Research Support: Society for Endocrinology Early Careers Grant awarded to ELT. The Department is funded by an Integrative Mammalian Biology (IMB) Capacity Building Award, an FP7-HEALTH-2009-241592 EurOCHIP grant and funding from the NIHR Biomedical Research Centre Funding Scheme.

Nothing to Disclose: MLS, ELT, KEB, JEB, SRB, KGM

Pub # P2-442

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Sex & Adipose Tissue Distribution (1:30 PM-3:30 PM)

Title Diet-Induced Obesity Is Associated with Sex-Specific Differences in Glucocorticoid Metabolism

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Body Whilst obesity affects both sexes the risk of associated metabolic disturbance is greater in men. Studies in animal models, mainly in males, suggest altered glucocorticoid metabolism contributes to the metabolic consequences of obesity. We hypothesised the differential response to diet-induced obesity in males and females would be accompanied by sex differences in glucocorticoid metabolism. Male and female C57Bl/6 mice were fed control (CON) or obesogenic (DIO) diets from 5 weeks of age (N=8/group). Mice underwent metabolic testing (glucose tolerance testing and diurnal blood sampling) at 13 weeks. The expression of glucocorticoid metabolising enzymes 11 β hsd1 (which reactivates glucocorticoids from their inert 11-keto metabolites) and 5 β r (glucocorticoid metabolism) was studied by real time PCR in liver and subcutaneous adipose (SC). DIO mice (male DIO 32.8g \pm 1.0g, female DIO 25.4 \pm 0.7g,) were heavier than CON (male 27.4 \pm 0.6g, P<0.001; female 22.2 \pm 0.6g p<0.01). We observed a sexually dimorphic pattern of gene expression in CON animals: in females, SC 11 β hsd1 expression was lower than in males (p<0.001) whereas hepatic expression of 11 β hsd1 was higher (p<0.05) predicting increased hepatic glucocorticoid regeneration. This is potentially offset in females by increased hepatic 5 β r expression (p<0.001). DIO induced sex-specific changes in glucocorticoid metabolism, increasing hepatic 11 β hsd1 (2.7 fold) and 5 β r (1.6 fold) and reducing SC 11 β hsd1 expression in males. In contrast in DIO females there were no changes in hepatic 11 β hsd1 or 5 β r expression but SC 11 β hsd1 expression was reduced to undetectable levels. There were also sex-differences in the metabolic response to DIO with marked fasting hyperglycaemia and hyperinsulinaemia in DIO males; while DIO females were only insulin resistant following a glucose load. In conclusion, DIO did not induce alterations in hepatic glucocorticoid metabolism in females, in association with a less severe metabolic phenotype. Thus, sexually dimorphic patterns of glucocorticoid metabolism may play a role in the differential metabolic responses to obesity in males and females. Lower adipose 11 β hsd1 coupled with higher hepatic A-ring reductase expression and/or the lack of change in response to DIO in females may partially protect them from the development of obesity and its metabolic sequelae.

Disclosures: BRW: Investigator, Wyeth Pharmaceuticals; Ad Hoc Consultant, Boehringer Ingelheim; Astra Zeneca. Nothing to Disclose: RSD, JRS, PWFH, AJD

Pub # P2-443

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Sex & Adipose Tissue Distribution (1:30 PM-3:30 PM)

Title Developmental Programming: Adipose Tissue Distribution in Prenatal Testosterone-Treated Sheep

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Body Prenatal testosterone (T) excess leads to a polycystic ovarian syndrome (PCOS)-like phenotype. PCOS affects approximately 10% of reproductive-aged women, of which 50-60% are overweight or obese. Obesity increases cycle irregularities, risk for infertility as well as miscarriage. Consistent with this, excess weight gain amplifies cycle disruptions in prenatal T-treated sheep. Prenatal T-treated sheep are also characterized by reduced insulin sensitivity; a trait found in ~70% of women with PCOS with excess weight gain amplifying degree of insulin resistance. Considering that visceral adiposity is evolving as a risk factor for the development of insulin resistance, the aim of this study was to determine if prenatal T excess increases visceral adiposity. We tested the hypothesis that the prenatal T-treated sheep will have a higher ratio of visceral to subcutaneous fat relative to control females. Pregnant sheep were given T propionate (100 mg, i.m twice weekly) in cottonseed oil from days 30-90 of gestation (term: ~147 d). To determine the effects of prenatal T treatment on adipose tissue distribution in the adult females, computed tomography (CT) was performed on control (C: n=6) and prenatal T-treated (T: n=6) females at 17 months of age prior to the second breeding season. Image analysis software (Analyze 6.0, AnalyzeDirect) was used to identify adipose tissue by means of an algorithm based on tissue density. Differentiation between subcutaneous and visceral compartments was based on manual tracing. Mean weight of animals at the time of scan was similar between groups (C: 76.9±0.9 vs. T: 76.0±2.7 kg). Mean total fat volume (C: 8.7±0.6 vs. T: 5.9±0.7 dm³; P<0.05) and visceral fat (C: 5.3±0.3 vs. T: 3.4±0.4 dm³; P<0.05) but not subcutaneous fat were higher in C females. The ratio of visceral to subcutaneous fat tended to be higher in C females (C: 1.60±0.05 vs. T: 1.37±0.12; P=0.07). The fact that prenatal T-treated females had less visceral fat compared to controls is consistent with our premise that the scans were undertaken during the compensatory response period similar to that seen with the insulin receptor signaling pathway in the liver/skeletal muscle (1), as an adaptive mechanism of the organism to restore homeostasis. It remains to be determined if visceral adiposity develops as the prenatal T-treated females advance in age.

(1) Nada et al., 2009 Society Study Reproduction P342, p125.

Sources of Research Support: NIH P01 HD44232.

Nothing to Disclose: VP, DP, AV-L, AP, JK

Pub # P2-444

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Sex & Adipose Tissue Distribution (1:30 PM-3:30 PM)

Title Testosterone Stimulation of Insulin-Sensitive Glucose Uptake in 3T3-L1 Adipocytes by Modulation of AMP-Kinase

Author String WE Taylor, A Dey, S Shirazi, R Singh
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Body Diabetes two is a serious disease, correlated with increased fat mass and decreased glucose uptake into cells o insulin resistance. In men, lower serum testosterone (T) is associated with increased type 2 diabetes, metaboli syndrome and obesity. However, T treatment stimulates increased insulin sensitivity and glucose uptake in cells, while decreasing fat mass in humans and mice. We previously showed, using 3T3-L1 adipocyte cultures, that treatment of fat cells with T stimulated glucose uptake. Confluent 3T3-L1 cultures of pre-adipocytes were grown in AM medium (containing insulin, dexamethasone, and IBMX) for 3 days and GM + insulin for 2 days to induce differentiation, then cells were treated in low-glucose (5 mM) growth medium (GM) with or without hormones T and insulin for various times. We measured glucose uptake in low-glucose media containing ³H-2-deoxy-glucose, and found that treatment with T (100 nM) for 1 to 6 hours increased 1 hr insulin-stimulated glucose uptake by about 30% in 3 hours. This stimulation was blocked by androgen receptor (AR) antagonist bicalutamide, thus the effect of T is mediated by AR. The mechanism by which T stimulates increased glucose uptake is not well understood. One hypothesis is that T may stimulate the insulin signaling pathway mediated by PI3K for activating phosphorylation of Akt1, leading to activation of the glucose transporter protein GLUT4 for glucose import into the cell. However, we showed that insulin signaling in 3T3-L1 adipocytes is not stimulated significantly by androgens, by doing Western immunoblotting of cell protein extracts with antibodies to Akt1, Phospho-Akt1 (Thr308, or Ser473), and GLUT4. We now hypothesize that Testosterone stimulation of GLUT4-mediated glucose uptake in 3T3-L1 adipocytes is mediated by regulation of AMP-dependent protein kinase (AMPK). Activation of P-AMPK by the drug AICAR is known to activate glucose uptake in muscle cells in response to low energy charge (high AMP) due to exercise or starvation. Conversely, in adipocytes, P-AMPK activation causes a decrease in glucose uptake. We observed that T caused a significant increase in P-AMPK in undifferentiated cells, but a decrease in P-AMPK in well-differentiated adipocytes stimulated by insulin. This would explain in part the observed increase of glucose uptake in adipocytes. If we can understand the pathways through which T increases glucose uptake, we can develop better treatments for the deadly disease of diabetes 2.

Nothing to Disclose: WET, AD, SS, RS

Pub # P2-445

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Sex & Adipose Tissue Distribution (1:30 PM-3:30 PM)

Title Androgens Modulate Osteocalcin Release by Visceral Adipose Tissue

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Body Obesity and cardiovascular diseases share clinical features such as altered insulin sensitivity, fasting glycemia lipid and hormonal pattern. Osteocalcin (OCN), traditionally known to be a bone-related marker as Gla-carboxylated protein (cOCN), has recently grown in interest for the protective role, exerted by its undercarboxylated form (ucOCN), in metabolic diseases. Recent studies performed in cohorts of obese patients documented that ucOCN/OCN ratio is negatively correlated to BMI and that both subcutaneous (SAT) and omental (OAT) adipose tissue express OCN and all genes involved in OCN Gla-carboxylation. Moreover both types of adipose tissue are able to release OCN in both its carboxylated and undercarboxylated form. Here we investigated in vitro the effect of androgen stimulation on OCN release by OAT, mainly involved in metabolic disorders. DHT stimulation for 24h was able to significantly increase the release of both ucOCN and cOCN by OAT at concentration of respectively 10^{-12} to 10^{-8} M and 10^{-10} to 10^{-8} M. This effect was androgen specific as evidenced by its abrogation in presence of an androgen receptor antagonist (flutamide). Moreover DHT enhanced the ucOCN/OCN ratio in a DHT concentration-dependent manner. We then evaluated 28 normal and 63 overweight-obese male subjects for plasma levels of cOCN, ucOCN, leptin, testosterone (total and free) and estradiol. As expected, we found that free testosterone was negatively correlated with BMI ($r = -0.706$, $P < 0.001$) whereas, interestingly, ucOCN/OCN ratio was significantly and positively correlated with free-testosterone ($r = 0.223$, $P < 0.05$). In conclusion our data strengthen the protective effect of androgens from metabolic disorders, by adding a new role on the modulation of the relative amount of ucOCN released by the adipose tissue.

Nothing to Disclose: LDT, AF, GS, LG, UC, AG, CF

Pub # P2-446

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Sex & Adipose Tissue Distribution (1:30 PM-3:30 PM)

Title Adipose Tissue Expression of Genes Coding for Prostaglandin Synthesis Enzymes and Receptors in Women

Author String A Michaud, S Noel, G Paris, V Luu-The, MA Fortier, A Tchernof
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Body **Introduction:** Series 2 prostaglandins (PGs) act as modulators of adipogenesis and lipolysis in adipose tissue **Objective:** We tested the hypothesis that adipose tissue expression of several genes coding for enzymes involved in series 2 PG synthesis or receptors are altered in women with abdominal obesity. **Methods:** Omental and subcutaneous fat samples were surgically-obtained in 49 women (age: 46.7±4.0 years, BMI: 27.9±8.5 kg/m²). Adipocyte diameter was measured in isolated mature fat cells. Body composition and fat distribution were measured by dual energy x-ray absorptiometry and computed tomography. Whole adipose tissue samples were used for mRNA abundance quantification of PTGS1 (cyclooxygenase 1), PTGS2 (cyclooxygenase 2), AKR1B1 and AKR1C3 (PGF synthases), PLA2G4 (phospholipase A2), PGIS (PGI synthase), PGDS (PGD synthase), PTGES3 (PGE synthase), PTGER3 and PTGER4 (PGE receptors) by realtime RT-PCR. **Results:** Visceral adipose tissue area was positively associated with omental adipose tissue mRNA abundance of PTGER3 (r=0.43), PTGER4 (r=0.31), as well as the ratio of AKR1C3/PTGES3 (r=0.48, p[le]0.05 for all), indirectly indicating increased synthesis of PGF2 α over PGE2. Subcutaneous adipose tissue area was positively correlated with subcutaneous mRNA abundance of PTGER4 (r=0.53) and the ratio of AKR1C3/PTGES3 mRNA (r=0.58, p[le]0.05 for all). Omental adipocyte diameter was significantly related to omental adipose tissue mRNA abundance of PTGER3 (r=0.51), PTGER4 (r=0.28), as well as the ratio of AKR1C3/PTGES3 mRNA (r=0.52, p[le]0.05 for all). Subcutaneous adipocyte diameter was significantly associated with subcutaneous adipose tissue mRNA ratio of AKR1C3/PTGES3 (r=0.50) and AKR1B1/PTGES3 (r=0.47, p[le]0.05 for both), indirectly suggesting increased PGF2 α synthesis. Omental adipocyte diameter was the best predictor of the AKR1C3/PTGES3 mRNA ratio in omental adipose tissue, explaining 27.1% of the variance in a model including subcutaneous adipocyte diameter, adipose tissue areas and total body fat mass. Subcutaneous adipose tissue area was the best predictor of the AKR1C3/PTGES3 mRNA ratio in subcutaneous adipose tissue, explaining 33.8% of the variance in a model including visceral adipose tissue area, adipocyte diameters and total body fat mass. **Conclusion:** Increased abdominal adiposity in women is associated with a shift in expression of PG synthesis enzymes that suggests higher PGF2 α synthesis over PGE2 in both the subcutaneous and omental fat compartments.

Nothing to Disclose: AM, SN, GP, VL-T, MAF, AT

Pub # P2-447

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Sex & Adipose Tissue Distribution (1:30 PM-3:30 PM)

Title Association of Serum Adiponectin and Postmenopausal Hypertension in Obese and Non-Obese Women

Author String S Faiz, Y Gebregiorgis, N Santanum, R Stanek, T Gress, A Yaquob
Marshall University, Joan C Edwards School of Medicine, Huntington, WV; Joan C Edwards School of Medicine, Huntington, WV

Body **Background:** Low circulating levels of Adiponectin have been associated with metabolic syndrome, diabetes and cardiovascular disease. Incidence of hypertension in women increases following menopause. Relationship between serum Adiponectin and post-menopausal hypertension has not been fully explored.
Hypothesis: We intended to study the association between hypertension, menopausal status and serum Adiponectin. We hypothesized that, after adjustment of BMI, post-menopausal women with hypertension will have lower Adiponectin levels than their normotensive pre-menopausal and post-menopausal counterparts.
Materials and Methods: We recruited 43 women in this cross sectional study conducted at Marshall University endocrinology clinic. Patients were stratified into 8 groups based on their menopausal status, BMI and presence of hypertension. Women with known diabetes, renal failure and cardiovascular disease were excluded. Serum total and high molecular weight (HMW) Adiponectin were measured by ELISA (ALPCO diagnostics, Salem, NH) and HMW-to-total Adiponectin ratio (HMWR) was calculated.
Results: Serum Adiponectin was significantly lower in obese as compared to non-obese women (P-value was <0.02 for total Adiponectin, <0.0017 for HMW Adiponectin and <0.002 for HMWR). Women with higher Waist-to-hip ratio (WHR) had significant trend towards lower total and HMW adiponectin levels as compared to those with lower WHR (P 0.014 for total Adiponectin & 0.04 for HMW). There was no significant difference in total or HMW Adiponectin levels among various patient groups. The difference in HMWR among various groups could be explained by obesity, being lower in obese groups as compared to non-obese groups.
Conclusion: We found that total, HMW Adiponectin and HMWR were significantly lower in obese women as compared to their non-obese counterparts. We also found that both total and HMW Adiponectin decreased with increasing WHR. We were unable to find any association between Hypertension, Menopausal status and serum Adiponectin.

Nothing to Disclose: SF, YG, NS, RS, TG, AY

Pub # P2-448

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Sex & Adipose Tissue Distribution (1:30 PM-3:30 PM)

Title Abdominal Obesity, Adipocyte Size and Reproductive History in Women

Author String JA Cote, A Veilleux, S Noel, A Tchernof
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Body Reproductive history events such as the timing of menarche or the number of pregnancies and parity have been related to obesity risk. However, little information is available on the link between reproductive events and measures of body fat distribution or abdominal adipose tissue cell size. **Objective:** To assess the association between age at menarche, number of pregnancies or parity and regional adiposity measures as well as adipocyte size in women. **Methods:** Omental and subcutaneous fat samples were surgically-obtained in 170 women (age: 47.1±5.1 years, BMI: 26.8±5.2 kg/m²). Adipocyte size was measured in cell suspensions of collagenase-digested tissues. Body composition and fat distribution were measured by dual energy x-ray absorptiometry and computed tomography respectively. The number of pregnancies, parity and the timing of menarche were obtained by questionnaire at the time of surgery. Parity and the number of pregnancies were examined in categories (0, 1, 2 and 3 or more). Women were also stratified in quintiles according to age at menarche. **Results:** No group difference was found among categories of parity or number of pregnancies in total body fat mass, visceral or subcutaneous adipose tissue areas and fat cell size. Mean age at menarche from the lowest to the highest quintile was 10.5±0.8, 12.0±0.0, 12.3±0.5, 13.4±0.5 and 15.1±1.1 years respectively. Women in the lowest quintile of menarche onset had on average 12.7% higher body weight (p<0.001) and 13.2% higher BMI values (p<0.0001) compared to women in all other quintiles. These results were independent of menopausal status and age of the women at testing. Women in the lowest quintile of menarche onset had a slightly but significantly higher (average 18.0%, p[le]0.01) total abdominal adipose tissue area compared to women in other quintiles, while there was no difference for visceral or subcutaneous areas examined separately. Omental adipocyte size was larger (average 10.2%, p<0.01) in women in the lowest quintile of menarche onset compared to all other quintiles. This was not observed for subcutaneous adipocyte size. Fasting insulin levels were significantly higher (average 27.1%, p<0.01) in women with early menarche (quintile 1) compared to others. However, this difference was no longer significant after adjustment for BMI. **Conclusion:** Our results provide evidence that early menarche is related to increased overall adiposity in women, and not specifically to body fat distribution measurements.

Nothing to Disclose: JAC, AV, SN, AT

Pub # P2-449

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Sex & Adipose Tissue Distribution (1:30 PM-3:30 PM)

Title Regional Differences in Adipocyte Populations of Human Abdominal Adipose Tissues

Author String M Nadeau, CO Soulage, HA Soula, A Geloën, H Vidal, C Bouchard, A Tchernof
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Body Osmium tetroxide fixation of adipose tissue generates a bimodal distribution of adipocyte sizes, with a population of large adipocytes and a proportion of small cells. Osmium-fixed adipocyte populations have never been compared in human omental (OM) and subcutaneous (SC) adipose tissues. **Objective:** To investigate omental and subcutaneous adipocyte size distributions in women covering the lean-to-obese range. **Methods:** OM and SC fat samples were obtained during gynecological surgery in 21 women (mean age: 46.3±4.6 years; mean BMI: 27.1±4.7 kg/m²). Samples were treated with osmium tetroxide and analyzed with a multisizer counter. Cell size distributions were computed for each sample with an exponential Gaussian fit. Body composition and fat distribution were measured by dual energy x-ray absorptiometry and computed tomography. **Results:** Bimodal distributions were found for all the samples examined. The mode of the population of large adipocytes (assessing median cell size of the large cell population) was significantly lower in OM compared to SC samples (93.9±20.0 vs. 114.8±12.7[μm], p<0.0001). The average nadir value of the distributions (indicating the least probable cell size) was also significantly lower in OM vs. SC fat sample: (52.0±14.9 vs. 59.7±9.4[μm], p<0.005). Small adipocytes had similar sizes among compartments as indicated by identical half-size (tau) values. However, the proportion of small to large cells was significantly lower in OM compared to SC samples (7.1±5.9 vs. 9.8±4.6%, p<0.01). Median size of the large adipocyte population (mode) in OM fat was positively related to total body fat mass (r=0.77, p<0.0001), SC (r=0.83, p<0.0001) and visceral (r=0.87, p<0.0001) adipose tissue areas. These correlations were also significant with median size of large SC cells (r=0.56, r=0.69 and r=0.67 respectively, p<0.01 for all). The proportion of small to large cells in OM samples was positively associated with body fat mass (r=0.63, p<0.002), SC (r=0.75, p<0.0001) and visceral (r=0.81, p<0.0001) adipose tissue areas. Associations of slightly lower magnitude were found with the proportion of small SC cells (r=0.48, 0.65, 0.61 respectively, p<0.05). **Conclusions:** OM adipocyte sizes have bimodal distributions, similar to that of SC cells. However, large OM fat cells have a lower median size and the proportion of small adipocytes is lower than in SC adipose tissue. Measures of overall and regional adiposity are strongly and positively related to these parameters.

Nothing to Disclose: MN, COS, HAS, AG, HV, CB, AT

Pub # P2-450
Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Sex & Adipose Tissue Distribution (1:30 PM-3:30 PM)
Title Human Visceral Preadipocytes Have a Greater Capacity To Transport Fructose Compared to Subcutaneous Preadipocytes
Author String G Coade, JMP Holly, JPH Shield, GI Welsh, EJ Foulstone
University of Bristol, Bristol, UK

Body
Background
In the USA over the last 10 years there has been a switch in sweeteners from sucrose to high fructose corn syrup, mirroring the rapid increase in obesity. This has resulted in a fructose intake over 5 times that of our ancestors, therefore extra-hepatic tissues e.g. fat may now be exposed to significantly raised levels. Previously we have found that exposure to fructose selectively increased the differentiation of visceral preadipocytes (VP). Here we investigated whether VP and subcutaneous preadipocytes (SCP) have different capacities to transport glucose and fructose resulting in differences in exposure.

Methods

Primary cultures of human preadipocytes were isolated from subcutaneous or visceral adipose tissue from prepubertal children. 2-Deoxyglucose uptake assays were performed with or without insulin (15 mins, 100nM) and fructose uptake was measured over 1h plus or minus cytochalasin B. GLUT1 and GLUT4 were assessed by Western blotting and GLUT5 by rtPCR.

Results

As GLUT4 was undetectable in the preadipocytes glucose uptake was concluded to be via GLUT1. Total GLUT1 levels were increased in SCP compared to VP (n=3). However, basal glucose uptake was unchanged between the two depots. Insulin increased glucose uptake suggesting translocation of GLUT1 to the plasma membrane but no difference was seen between VP and SCP. Overall fructose uptake was greater in SCP than VP despite higher expression of GLUT5 mRNA in VP (n=3). However inhibiting GLUT1, which transports both glucose and fructose, with cytochalasin B revealed a 2-fold increase in fructose transport in VP (p<0.05, n=3). In vivo fat cells will never be exposed to fructose alone, so we investigated uptake in the presence of glucose. As with GLUT1 inhibition, fructose uptake was reduced but with VP again transporting 2-fold more fructose than SCP (p<0.05, n=3).

Conclusion

Although VP and SCP both take up glucose to a similar extent, VP can transport more fructose in the presence of glucose due to increased GLUT5 expression. Therefore on fructose exposure, VP have a greater capacity to take up this sugar which by-passes the metabolic controls in place for glucose utilisation, resulting in an unlimited amount of raw material for triglyceride production. This may allow increased differentiation and lipid accumulation in visceral fat which is more closely associated with the morbid effects of obesity and the metabolic syndrome.

Sources of Research Support: Diabetes UK studentship.

Nothing to Disclose: GC, JMPH, JPHS, GIW, EJF

Pub # P2-451

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Sex & Adipose Tissue Distribution (1:30 PM-3:30 PM)

Title Subcutaneous and Visceral Preadipocytes Taken from Healthy-Weight Prepubertal Children Show Distinct Differences in Their Response to IGFs

Author String G Coade, JMP Holly, JPH Shield, GI Welsh, EJ Foulstone
University of Bristol, Bristol, UK

Body

Background
Visceral adiposity is more closely associated with the morbid effects of obesity and the metabolic syndrome than subcutaneous fat. In addition, preadipocytes isolated from the different depots have distinct characteristics with subcutaneous cells proliferating faster. Cells isolated from visceral tissue of obese adults show impaired IGF-I signalling which might contribute to this growth phenotype. Here we investigated how IGFs stimulate preadipocytes isolated from subcutaneous (SCP) or visceral (VP) adipose tissue taken from normal weight pre-pubertal children.

Methods
Preadipocytes were differentiated for 14 days with 100nM insulin or 100ng/ml IGF-I or II. 2-Deoxyglucose uptake assays were performed (100nM insulin, 15 mins). Phosphorylation of PKB was assessed by Western blotting and DNA synthesis by [³H]-thymidine uptake.

Results
IGF-I-stimulated PKB phosphorylation was maximal in both SCP and VP by 5-10 mins. However, in VP phosphorylation had begun to decrease after 30 mins whereas it was still maximal in SCP (n=3). In addition IGF-I and II were able to stimulate DNA synthesis in SCP but not in VP. Whilst preadipocytes have traditionally been considered to require insulin for differentiation, in the absence of insulin, IGF-I and II were able to stimulate preadipocyte differentiation. In VP differentiation was similar to that stimulated by insulin whereas in SCP there were fewer mature lipid containing fat cells indicating reduced differentiation (n=3). As a consequence insulin-stimulated glucose uptake in the mature adipocytes was unaffected in VP but significantly compromised in the SCP cultures (67% and 42% of control in cells differentiated with IGF-I and IGF-II respectively n=3 p<0.05).

Conclusion
Although phosphorylation of PKB by IGF-I was not reduced in our VP cultures, as had been reported in obese adult preadipocytes, activation was more transient and IGFs were unable to promote proliferation. Failure to sustain activity of this signalling pathway might contribute to the reduced proliferation of VP. To differentiate cells need to exit the cell cycle. IGFs did not stimulate VP to progress through the cell cycle which might help explain why IGFs were able to stimulate differentiation to a greater extent in these cultures compared to SCP. As IGFs can substitute for insulin in promoting differentiation of VP this may facilitate inappropriate increases in mature adipocytes in this fat depot.

Sources of Research Support: Diabetes UK studentship.

Nothing to Disclose: GC, JMPH, JPMS, GIW, EJJ

Pub # P2-452

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Sex & Adipose Tissue Distribution (1:30 PM-3:30 PM)

Title The Effect of Growth Hormone on Macrophage Content in Subcutaneous vs. Intra-Abdominal Adipose Depots

Author String R Munn, AJ Palmer, V Magon, EO List, JJ Kopchick, DE Berryman
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Body Obesity is characterized by a state of chronic, low-grade inflammation. This inflammation is linked with an increase in the number of macrophages present in adipose tissue. Under normal conditions, adipose tissue contains a resident population of macrophages that performs functions such as tissue remodeling and is designated as M2 (1). Under obese conditions, there is an accumulation of infiltrating macrophages that secrete pro-inflammatory molecules and are designated as M1 macrophages. Growth hormone (GH) has a profound effect on adipose tissue and has been shown to decrease the amount of fat mass and increase the amount of lean mass in mice in a depot specific manner. Previous studies also show that GH has a direct impact on macrophages, altering their morphology and expression profile. This suggests that in addition to having profound, direct effects on adipose tissue, GH may also act indirectly on adipose by altering the expression profile of macrophages within the tissue. In addition, macrophage infiltration has been shown in wild-type mice to differ depending on the adipose tissue depot. Thus, the purpose of this study is to assess the relationship between GH and macrophage phenotype in various adipose depots. This study examines the inguinal and epididymal adipose tissue of mice of three genotypes: bovine GH transgenic (bGH), GH receptor knockout (GHR^{-/-}) and growth hormone receptor antagonist transgenic (GHA) mice as well as their littermate controls. Expression levels of several M1 and M2 macrophage markers were determined by real-time PCR. These markers include: one general macrophage marker (emr1 - EGF-like module-containing mucin-like hormone receptor-like 1), four M1 markers (Tnf - tumor necrosis factor, Il6 - interleukin 6, Il1b - interleukin 1-beta, Ccl2 - monocyte chemoattractant protein-1), and four M2 markers (Arg1 - arginase 1, Il10 - interleukin 10, Retnla - resistin-like alpha, Chi3l3 - chitinase 3-like-3). Preliminary data suggest that the expression profile of macrophages within subcutaneous adipose tissue is more significantly altered by GH than those of epididymal adipose. Within subcutaneous adipose, GH causes the upregulation of both M1 and M2 macrophage markers. Additionally, the expression of these markers was shown to increase with age in wild-type mice. Further experimentation and data analysis will provide further insight into the relationship of GH and macrophage subtypes in different adipose tissue depots.

(1) Anderson EK et al., *Curr Opin Lipidol.* 2010 21:172-7.

Sources of Research Support: In part by the State of Ohio's Eminent Scholar Program that includes a gift from Milton and Lawrence Goll, by the AMVETS, by the Diabetes Research Initiative at Ohio University, and by NIH grants DK083729, AG019899, AG031736.

Nothing to Disclose: RM, AJP, VM, EOL, JJK, DEB

Pub # P2-453

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Sex & Adipose Tissue Distribution (1:30 PM-3:30 PM)

Title Effects of the Hypoxic Microenvironment on Stem Cells Isolated from Omental and Subcutaneous Adipose Tissue from Obese Patients

Author String L Salvatori, G Coroniti, F Caporuscio, L De Girolamo, D Stanco, G Silecchia, L Principessa, L Ravenna, MA Russo, AT Brini, E Petrangeli
CNR, Rome, Italy; Regina Elena Cancer Institute, Rome, Italy; Sapienza University of Rome, Rome, Italy; IRCCS Galeazzi Orthopedic Institute, Milan, Italy; University of Milan, Milan, Italy; Sapienza University of Rome, Rome, Italy

Body Omental and subcutaneous adipose tissues (AT) show a different inflammatory activity since the release of pro-inflammatory cytokines is much greater in omental fat. This activity depends on the presence of preadipocytes, mature adipocytes and macrophages and involves autocrine and paracrine mechanisms which contribute to the amplification of signals. Moreover, AT of obese patients shows a reduced pressure of oxygen that could be involved in the expression of pro-inflammatory genes.
The aim of our study is to characterize subpopulations of adipose stem cells (hASC) isolated from omental and subcutaneous adipose tissue from normal and obese patients in order to evaluate possible differences in the expression of the inflammatory phenotype. In particular, cells isolated from normal donors represent the control against which we will evaluate hASC of obese patients. To analyse the expression of inflammation-related genes we perform Real Time PCR using specific TaqMan Gene Expression Arrays realized on our design that allow us to study 48 genes at the same time.
Our data indicate that hASC isolated from the four adipose tissues analyzed show different morphological and functional features. In fact, the clonogenic potential of cell populations, evaluated by Fibroblast-Colony Forming Unit (CFU-F) assay, was strongly higher in hASC isolated from normal patients than in obese ones and higher in subcutaneous than in omental tissue. These differences were confirmed by the analysis of proliferation rates of the four subpopulations. Since the major functional differences were observed in hASC isolated from subcutaneous tissue of normal donors and from omental tissue of obese patients, we have evaluated the effects of hypoxic treatment on these two cell subpopulations. Preliminary data suggested that both cell types were responsive to hypoxic environment resulting in the activation of pro-inflammatory genes. However, the modulation of gene expression is quite different in the two cell subpopulations. Defining the effects of hypoxia on hASC can be useful to the identification of novel therapeutic targets in order to heal obesity.

Nothing to Disclose: LS, GC, FC, LDG, DS, GS, LP, LR, MAR, ATB, EP

Pub # P2-454

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Sex & Adipose Tissue Distribution (1:30 PM-3:30 PM)

Title Depot-Specific Differences in the Sensitivity to Glucocorticoid and Insulin Action in Human Adipose Tissue

Author String LL Gathercole, SA Morgan, IJ Bujalska, PM Stewart, JW Tomlinson
University of Birmingham, Birmingham, UK

Body Intra-abdominal adiposity is associated with insulin resistance, Type 2 Diabetes and increased cardiovascular morbidity and mortality. Differences in gene expression between omental (om) and subcutaneous (sc) adipose have been described, however the molecular mechanisms that underpin these differences in adipose biology are not known. Patients with glucocorticoid excess, Cushing's syndrome, develop a phenotype characterized by central obesity. We have characterized the regulation of lipogenesis by glucocorticoids and insulin in paired om and sc differentiated pre-adipocytes measuring lipogenic gene expression by real-time PCR and 1-[14C] -acetate incorporation into lipid.

Differentiated sc pre-adipocytes had more lipid droplets, increased mRNA expression of lipogenic genes (ACC1 3.2 fold, FAS 10.9 fold, ACC2 17.8 fold, LPL 44.7 fold) and higher 1-[14C]-acetate incorporation than om cells (8.9 fold). However, lipogenesis in om cells was more responsive to insulin stimulation (control [100%]: sc 149±22.14%, om 209±1.86%, p<0.05). In the absence of insulin, Dexamethasone (Dex) had no effect on lipogenic gene expression in sc or om cells, however, decreased lipogenesis in both sc (100% [control], 81.9±3.9%[5nM], 67.3±4.8%[500nM]) and om (72.0±5.4%[5nM], 46.9±5.7%[500nM]) cells in a dose dependent manner. Interestingly in the presence of insulin, in both sc and om cells, Dex increased FAS expression, (sc 2.4 fold; om 3 fold) and in om cells only, increased ACC1 expression (1.6 fold). In sc cells, in the presence of insulin (5nM), Dex no longer inhibited lipogenesis and did not impair insulin-stimulated lipogenesis (100%[control], 149.0±22.1%[No Dex], 145.5±29.4%[5nM], 128.2±39.3%[500nM]). In contrast, Dex decreased lipogenesis in om cells in the presence of insulin, reflecting either a persistence of the direct action of Dex, or its ability to induce insulin resistance and limit insulin-stimulated lipogenesis (100% [control], 208.9±1.86[No Dex], 181.45±11.8%[5nM], 125.1±12.0%[500nM]).

Om adipose tissue sensitivity to both glucocorticoids and insulin may drive lipid flux, contributing to the Cushing's phenotype and may also help to explain the detrimental impact of om fat accumulation.

Nothing to Disclose: LLG, SAM, IJB, PMS, JWT

Pub # P2-455

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Sex & Adipose Tissue Distribution (1:30 PM-3:30 PM)

Title Differential Abundance of Proteins in Omental and Subcutaneous Adipose Tissue

Author String ML Insenser, RL Montes, RL Simo, JL Vendrell, JL San-Millan, HF Escobar-Morreale
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Body Background: At present, visceral adipose tissue is considered a highly active metabolic and endocrine organ and not a mere energy-storage depot. The proteomic approach to the study of visceral fat has the potential advantage of providing a global vision of the cellular pathways associated with the disease under study, permitting the identification of novel candidate molecules that would have not been considered of interest otherwise.

Objective: The aim of the present study is to identify novel proteins showing different abundance in subcutaneous and omental adipose tissue by applying two-dimensional fluorescence difference gel electrophoresis to adipose tissue samples.

Patients and Methods: Paired samples of omental and subcutaneous adipose tissue were obtained from 26 Caucasian men and 9 Caucasian women who had been submitted to elective surgical procedures including cholecystectomy, surgery of abdominal hernia and gastric bypass surgery. Obesity was defined by a body mass index (BMI) above 30 kg/m². Paired subcutaneous and omental fat samples from 12 patients were included in the present study: six non-obese patients (3 male and 3 female, aged from 21 to 76, body mass index 24.5 ± 2.2 kg/m²) and six obese patients (3 male and 3 female, aged from 18 to 59, BMI 51.8 ± 8.4 kg/m²).

Proteins from subcutaneous and omental tissue samples were extracted by using a Polytron PT-1200C homogenizer directly in disruption buffer. Interfering components were removed using the 2D Clean Up Kit and proteins were resuspended in a proper buffer. The samples were analyzed by two-dimensional difference in gel electrophoresis (2D-DIGE). Protein spots showing significantly different abundance among groups were identified, excised, trypsin digested and analyzed by matrix-assisted laser-desorption ionization time-of-flight time-of-flight (MALDI-TOF-TOF).

Results: This approach allowed the analysis of approximately 1296 protein spots in the comparative study of proteins, revealing 7 protein-spots with statistically different abundance levels. The identification of proteins is undergoing, as is the validation of identified spots by western blot and RT-PCR.

Conclusions: There are differences in protein abundance among adipose tissue depots, and such differences are also influenced by the presence or absence of obesity. Full identification of the proteins involved may reveal novel proteins involved in the pathophysiology of obesity and associated conditions.

Sources of Research Support: Grants FIS PI 050341, REDIMET RD06/0015/0007, and CIBER Diabetes y enfermedades metabólicas asociadas (CIBERDEM) CB07/08/0005 from the Spanish Ministry of Science and Innovation, Instituto de Salud Carlos III.

Nothing to Disclose: MLI, RLM, RLS, JLV, JLS-M, HFE-M

Pub # P2-456

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Sex & Adipose Tissue Distribution (1:30 PM-3:30 PM)

Title Plasma Annexin A1 (AnxA1): A Possible Biomarker of Central Fat Mass in Humans

Author String A Kosicka-Knox, A Cunliffe, R Mackenzie, R Fowkes, R Flower, D Renshaw
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Body Recent studies suggest that obesity related metabolic disorders are characterised by chronic mild inflammation as a result of adipocytokine production from fat tissue leading to dysregulation in the pro/anti-inflammatory systemic balance. Adipokines and pro-inflammatory markers are implicated in insulin insensitivity, blood glucose dysregulation, inflammation and atherosclerosis. These immunoactive molecules provide a molecular link between excess adiposity and the development of type 2 diabetes and cardiovascular disease. As a result, circulating levels of adipokines and pro-inflammatory markers may be valuable biomarkers in identifying future risk of a range of disease states associated with obesity. Whilst there is a considerable amount of research into the characterisation of adipokines and pro-inflammatory cytokines, the anti-inflammatory mediators have received little attention.

AnxA1, is an endogenous glucocorticoid regulated protein, which mediates systemic anti-inflammatory processes. The expression of AnxA1 with increased adiposity has not been investigated to date. However, given the balance that exists between pro and anti-inflammatory substances, it seems plausible that AnxA1 may be altered in individuals with increasing adiposity.

We recruited 93 healthy males for an *in vivo* study measuring both metabolic and anthropometric parameters. AnxA1 was measured using an in-house sandwich ELISA. Adipocytokines were measured using DuoSet ELISA kits. Ethical approval was granted by the University of Westminster (08/09/22). Plasma ANXA1 levels were correlated with level of adiposity and distribution of fat, Body Mass Index (BMI), waist to hip ratio (WHR), % body fat (measured via Bod Pod, Life Measurements, Inc.), blood pressure, fasting blood cholesterol and glucose, insulin and cortisol. Standard adipocytokines were also measured including adiponectin, leptin, IL-6, IL-10 and TNF α .

As others have previously demonstrated, leptin and adiponectin were significantly correlated with WHR ($p < 0.001$, $R = 0.706$ and $p = 0.021$, $R = -0.225$, respectively). Interestingly, plasma AnxA1 protein was significantly inversely correlated with WHR ($P=0.017$) in healthy male subjects suggesting that as central fat mass increases the concentration of plasma AnxA1 decreases.

Plasma AnxA1 could represent a depot specific biomarker whose decline with increasing central adiposity may relate to the phenomena of increasing systemic inflammation and associated disease risk.

Sources of Research Support: Dr D Renshaw is supported by an Early Career grant from the Society for Endocrinology; A Kosicka-Knox was supported by a 2 year scholarship from the Human and Health Sciences Department, School of Life Sciences, University of Westminster.

Nothing to Disclose: AK-K, AC, RM, RF, RF, DR

Pub # P2-457

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Sex & Adipose Tissue Distribution (1:30 PM-3:30 PM)

Title Subcutaneous and Visceral Adipose Tissue Expression of EHD1, a Protein Involved in GLUT4 Translocation Correlates to Clock Gene Expression, Especially in Type 2 Diabetic Patients

Author String MM Zanquetta, ML Correa-Giannella, D Giannella-Neto, PA Alonso, LMMV Guimaraes, DM Bellandi, A Meyer, SMF Villares
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Body **Aims:** Disrupted circadian rhythms are associated with diabetes mellitus and metabolic alterations. Adipocytes possess an intrinsic circadian clock mechanism and rhythms in adipose tissue metabolism are well established. EHD1 is a key protein that regulates recycling and translocation of GLUT4. The aim of the present study was to analyze RNA expression of clock genes, *GLUT4* and *EHD1* in subcutaneous (SAT) and visceral (VAT) adipose tissues of non-diabetic (ND) and type 2 diabetic (D) patients as well as correlations among gene expressions and influence of the diabetic state.

Methods: SAT and VAT samples were obtained from 53 obese patients (40±11 years; 34.1±9 kg/m²; 32ND and 21D). Adipose tissues were collected from 12AM to 5PM. Glycemia, total cholesterol, HDL, LDL, VLDL and triglycerides were determined in the fasting state. Arterial systolic and diastolic blood pressures (BP) were also measured. *CLOCK*, *BMAL1*, *CRY1*, *PER1*, *PER2*, *REVERBa*, *EHD1*, *GLUT4* and *GAPDH* expressions were evaluated by qRT-PCR using fluorescent-labeled probes (TaqMan, Applied Biosystems). Non-parametric tests were used to compare ND and D groups and Spearman's Rho was employed to define correlations.

Results: D showed higher glycemia in comparison to ND patients (D: 109.8±16.9 mg/dL; ND: 88.3±6.7 mg/dL; $P<0.0001$). BMI, BP and the other biochemical measurements were not different between ND and D patients. *GLUT4* expression was significantly reduced in SAT (44% lower) and VAT (32% lower) of D in comparison to ND patients ($P<0.01$). *PER1* and *PER2* expressions were lower in SAT (43% and 30%, respectively) of D patients. In ND patients, SAT *EHD1* expression correlated to *PER1* ($r=0.66$; $P=0.0004$), *PER2* ($r=0.53$; $P=0.006$) and *CRY1* ($r=0.65$; $P=0.0003$); in VAT, *EHD1* correlated to *CLOCK* ($r=0.55$; $P=0.003$), *PER1* ($r=0.58$; $P=0.002$) and *CRY1* ($r=0.69$; $P=0.0001$). In D patients, these correlations were intensified and new ones were found: SAT *EHD1* correlated to *CLOCK* ($r=0.63$; $P=0.01$), *PER1* ($r=0.73$; $P=0.0003$) and *CRY1* ($r=0.95$; $P<0.0001$); VAT *EHD1* correlated to *CLOCK* ($r=0.74$; $P=0.0004$), *PER1* ($r=0.67$; $P=0.004$), *PER2* ($r=0.66$; $P=0.005$), *CRY1* ($r=0.74$; $P=0.0005$) and *REVERBa* ($r=0.69$; $P=0.001$).

Conclusions: *EHD1* RNA expression correlates to clock genes expression in SAT and VAT, especially in diabetic patients. These results can help to explain impaired *GLUT4* expression and translocation in the diabetic state.

Sources of Research Support: FAPESP-Fundacao de Amparo a Pesquisa do Estado de Sao Paulo #2008/05601-4 and #2009/03065-0.

Nothing to Disclose: MMZ, MLC-G, DG-N, PAA, LMVG, DMB, AM, SMFV

Pub # P2-458

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Sex & Adipose Tissue Distribution (1:30 PM-3:30 PM)

Title BMI Influence in Subcutaneous and Visceral Adipose Tissues Clock Gene Expression Depends on Gender

Author String MM Zanquetta, ML Correa-Giannella, D Giannella-Neto, PA Alonso, LMMV Guimaraes, DM Bellandi, A Meyer, SMF Villares
University of São Paulo Medical School, São Paulo, Brazil; University of São Paulo Medical School, São Paulo, Brazil; Edmundo Vasconcelos Hospitalar Complex, São Paulo, Brazil

Body

Aims: Disrupted circadian rhythms are associated with obesity and metabolic alterations. Adipocytes possess an intrinsic circadian clock mechanism and rhythms in adipose tissue metabolism are well established. It is known that body weight is correlated to clock genes expression (1,2). The aim of the present study was to analyze RNA expression of clock genes in subcutaneous (SAT) and visceral (VAT) adipose tissues of male and female subjects in the morning (AM) and afternoon (PM) periods, as well as its interactions with BMI.

Methods: SAT and VAT samples were obtained from 92 patients (40±12 yrs; 27 men and 65 women) into two periods of the day: AM (8h-12h) or PM (12h-17h); body mass index (BMI) ranged from 18.9-50.7 kg/m². *CLOCK*, *BMAL1*, *CRY1*, *PER1*, *PER2*, *REVERBa* and *GAPDH* RNA expressions were evaluated by qRT-PCR using fluorescent-labeled probes (TaqMan, Applied Biosystems). Non-parametric tests were used to compare groups and Spearman[acute]s Rho was employed to define correlations.

Results: Clock genes showed different patterns of expression between the day periods; percentages of change in PM in relation to AM period were: (1) female: *VATBMAL1* (80% higher; $P<0.001$), *SATBMAL1* (75% higher; $P<0.01$), *VATPER1* (47% lower; $P<0.01$), *SATPER1* (38% lower; $P<0.05$), *VATPER2* (32% lower; $P<0.01$), *SATPER2* (23% lower; $P<0.05$), *VATREVERBa* (57% lower; $P<0.01$), *SATREVERBa* (45% lower $P<0.05$) and (2) male: *VATBMAL1* (136% higher in PM; $P<0.05$), *VATPER1* (38% lower in PM; $P<0.05$), *SATPER1* (51% lower in PM; $P<0.01$), *VATREVERBa* (71% lower in PM; $P<0.05$), *SATREVERBa* (83% lower in PM; $P<0.05$). The comparison of RNA expressions between male and female subjects in the AM and PM periods separately demonstrated no significant differences. In female subjects, BMI was negatively correlated to *SATPER1* ($r=-0.60$; $P=0.011$) and *SATPER2* ($r=-0.58$; $P=0.014$) in the AM period; in the PM period, BMI was positively correlated to *VATCLOCK* ($r=0.46$; $P=0.004$) and negatively to *SATPER2* ($r=-0.47$; $P=0.006$). Unexpectedly, no correlations of BMI with *clock genes* expression were found in male subjects in any of the periods of the day.

Conclusions: The pattern of RNA expression of the clock genes differs between the AM and PM periods both in SAT and VAT in male and female subjects. The influence of BMI in clock genes expression seems to depend on gender.

- (1) Kaneko K et al., Brain Res 2009; 1263:58
(2) Kohsaka A et al., Cell Metab 2007; 6(5):414

Sources of Research Support: FAPESP-Fundacao de Amparo a Pesquisa do Estado de Sao Paulo #2008/05601-4 and #2009/03065-0.

Nothing to Disclose: MMZ, MLC-G, DG-N, PAA, LMVG, DMB, AM, SMFV

Pub # P2-459
Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Sex & Adipose Tissue Distribution (1:30 PM-3:30 PM)
Title Effects of Dietary Macronutrient Composition on Total and Intra-Abdominal Adipose Tissue during Weight Maintenance and Weight Loss

Author String LL Goree, A Ellis, P Chandler-Laney, K Casazza, B Gower
The University of Alabama at Birmingham, Birmingham, AL

Body BACKGROUND: Reduced dietary carbohydrate (CHO) and glycemic load (GL) may reduce insulin secretion and thereby facilitate loss of adipose tissue. OBJECTIVE: We tested the hypothesis that a consumption of a reduced CHO (RedCHO)/higher fat diet vs a standard (STD) diet would reduce insulin secretion, thus resulting in less intra-abdominal adipose tissue (IAAT) during weight maintenance and less total body adiposity during weight loss. METHODS: Sixty-nine healthy overweight men and women (36 European Americans (EA), 33 African Americans) were provided food for two consecutive 8 week diet intervention phases: eucaloric and hypocaloric (1000 kcal/day deficit). Participants received either a RedCHO/higher fat diet (N=40; 43% CHO, 18% protein, 39% fat) or STD diet (N=29; 55% CHO, 18% protein, 27% fat). The RedCHO/higher fat diet contained foods with a relatively low GL ([le] 45 points/1000 calories). Body composition and fat distribution (IAAT) were assessed before and after each phase by DXA and CT, respectively. Before and after completion of the eucaloric phase, insulin secretion was assessed in response to a standardized liquid meal (59% CHO, 17% protein, 24% fat) and during one solid breakfast meal (RedCHO/higher fat or STD). RESULTS: After completion of the eucaloric phase, both acute and chronic insulin secretion were lower with the RedCHO/higher fat diet (P<0.05 for both). Participants who consumed the RedCHO/higher fat diet had 11% less IAAT than those who consumed the STD diet (P<0.05, adjusted for total fat mass and baseline IAAT) after the eucaloric phase. In subgroup analysis, we found this loss of IAAT was specific to EA (P<0.05). After the hypocaloric phase, participants who consumed the RedCHO/higher fat diet had 4% less total fat mass than those who consumed the STD diet (P<0.05, adjusted for lean mass and baseline fat mass). No differences were found in IAAT (P=0.158) nor lean mass (P=0.367) at completion of the hypocaloric phase. CONCLUSION: Consumption of a RedCHO/higher fat diet, relative to a STD diet, promoted loss of IAAT during weight maintenance and augmented loss of total body fat during weight loss.

Sources of Research Support: National Institute of Health and National Institute of Diabetes and Digestive and Kidney Disease (R01DK67538). Core laboratory support, nursing, and inpatient/outpatient facilities were provided by M01-RR-00032 (GCRC), UL1RR025777 (CTSA), P30-DK56336 (NORC), and P60DK079626 (DRTC).

Nothing to Disclose: LLG, AE, PC-L, KC, BG

Pub # P2-460

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Sex & Adipose Tissue Distribution (1:30 PM-3:30 PM)

Title Depot-Specific Adipose Weight and Gene Expression Is Modulated by Myostatin and Activin

Author String L Bonomi, N Ungerleider, JE Silva, S Sen, ML Brown, A Schneyer
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Body Members of the TGF β superfamily, including myostatin (MSTN) and activin, have emerging roles in regulating glucose homeostasis and adiposity. Both MSTN and activin promote preadipocyte proliferation and inhibit differentiation into mature adipocytes *in vitro* while transgenic expression of MSTN in preadipocytes produces smaller adipocytes with enhanced insulin sensitivity and glucose uptake. MSTN and activin bioactivity is regulated by the antagonists follistatin (FST) and follistatin like-3 (FSTL3/FLRG). FST secretion from human adipocytes is reduced in obese patients and FSTL3 and myostatin biosynthesis is increased in obese rodent models. We previously reported that deletion of FSTL3 resulted in enhanced glucose tolerance and insulin sensitivity in mice, as well as reduced gonadal fat pad weight, consistent with the view that decreased visceral fat enhances insulin sensitivity. We recently described a new mouse model in which only one of the three FST protein isoforms were expressed (FST288-only). We also created a double mutant (2xM) by crossing these lines. To explore the roles of MSTN and activin in regulating adiposity we examined 9 month old males of each genotype for fat pad weight, whole body adiposity (qNMR) and gene expression (qPCR). In contrast to the single mutants, 2xM males have increased whole body adiposity as well as relative insulin resistance and glucose intolerance, although body weight is not significantly different from the other lines. Like FSTL3 KO males, gonadal fat pad weight was significantly reduced in 2xM mice. In contrast, mesenteric fat mass was significantly increased in all 3 lines while no significant differences in perirenal fat were observed for any line. Subcutaneous fat mass (flank and scapular) was significantly increased in 2xM males relative to FST288-only and WT mice, but not different from FSTL3 KO or WT mice, while brown fat was significantly increased in FST288-only males relative to WT littermates. FAS expression was significantly reduced in 2xM males, and inflammatory gene expression (MCP1, IFN γ) was increased in 2xM flank and gonadal depots. These results suggest a link between inflammatory gene expression and a switch from enhanced whole body insulin sensitivity (FSTL3 KO) to insulin resistance (2xM). Since these mouse models represent differentially increased MSTN and activin bioactivity, our results suggest that these growth factors regulate adipogenesis in a depot-specific manner.

Sources of Research Support: NIH/NIDDK Grant DK075058 to AS.

Nothing to Disclose: LB, NU, JES, SS, MLB, AS

Pub # P2-461

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Sex & Adipose Tissue Distribution (1:30 PM-3:30 PM)

Title Feeding Out of Diurnal Phase Changes Body Composition and Induces Depression in Rats

Author String JP Dunn, S Dosovitz, KA Rogers, JN Lukasiewicz, S Saadat, KD Niswender
Vanderbilt University School of Medicine, Nashville, TN

Body Depression is a common co-morbidity of obesity, while as many as 40% of patients with obesity have Night Eating Syndrome (NES). NES is associated with poor mental health outcomes including decreased response to first line therapeutics. We hypothesized that diurnal eating behaviors influence body weight/composition and depressive response to the forced swim test (FST). Long Evans Rats (n=16) were separated into 2 groups, exposed to a 45% high fat diet (HFD) restricted either to the light cycle (light restricted, LR) or restricted to dark cycle (dark restricted, DR) on a 12 hour lights on/off cycle. Body weight, body composition, and forced swim test (FST) responses were assessed at regular intervals. Baseline body weights were similar in LR and DR rats (284 ± 3 g for both groups). By day 4, the LR weighed less than DR rats (283 ± 4 g vs. 299 ± 4 g, $p=0.011$) and continued to weigh less at each time point thereafter: day 14 330 ± 5 g vs. 354 ± 6 g, $p=0.008$, and day 21 342 ± 6 g vs. 372 ± 8 g, $p=0.010$. The LR rats ate less than the DR rats over the course of the study (1575 ± 33 kcal vs 1853 ± 46 kcal, $p=0.004$). Baseline lean and fat mass were similar in LR and DR rats. While fat mass in both groups was similar at all time points (ANOVA $p=0.655$, day 21 59 ± 4 vs 61 ± 4 , LR vs DR, respectively), LR rats had significantly lower lean mass: day 4 219 ± 4 g vs 233 ± 4 g, $p=0.030$, day 14 239 ± 6 g vs. 262 ± 4 g, $p=0.007$, and day 21 245 ± 6 g vs. 272 ± 5 g, $p=0.004$. Time spent struggling (active swim) during FST was shorter, reflecting increased depressive-like behavior, in the LR compared to DR rats on day 15 (68 ± 17 sec. vs 108 ± 18 sec., $p=0.132$) and day 21 (71 ± 11 sec. vs 137 ± 20 sec., $p=0.010$). Despite a smaller gain in total body mass, rats restricted to HFD during their inactive phase reveal a body composition of excessive adipose relative to lean mass, suggesting a relative increase in partitioning of calories to adipose tissue and reduced partitioning to lean mass. LR fed rats also displayed increased depressive-like behavior compared to rats with access to HFD only during their active phase, suggesting either that HFD during inactive phase promotes depression or HFD restricted to active phase protects from depression. Future studies are needed to discern the importance of diurnal eating behaviors in obesity and depression.

Sources of Research Support: NIDDK (DK085712).

Nothing to Disclose: JPD, SD, KAR, JNL, SS, KDN

Pub # P2-462

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Sex & Adipose Tissue Distribution (1:30 PM-3:30 PM)

Title Tissue-Specific Changes in Genes Regulating Glucocorticoid Metabolism in Mice with Diet-Induced Obesity

Author String E Harno, A Yu, AV Turnbull, B Leighton, A White
University of Manchester, Manchester, UK; AstraZeneca, Alderley Park, UK

Body Increased circulating glucocorticoids are associated with central obesity and metabolic syndrome, but how this affects glucocorticoid metabolism is unclear. We aim to determine how obesity affects circulating HPA axis biomarkers and genes regulating corticosterone metabolism in liver and adipose tissues. These genes include 11 β -HSD1, which regenerates corticosterone from its inactive form 11-dehydrocorticosterone (11-DHC), 5 α -reductase and 5 β -reductase which increase clearance of corticosterone and 11 β -HSD2, which converts corticosterone to 11-DHC.

Male C57Bl6/Jax mice were fed 60% fat by energy diet for 13 weeks and then plasma, liver and epididymal adipose tissue collected at 5 times around the expected peak and nadir of corticosterone (n=6). Plasma biomarkers were measured by ELISAs and gene expression was determined by Taqman [ldquo]Assay on Demand[rdquo] probes.

Obese mice had increased circulating corticosterone and POMC, indicative of an upregulated HPA axis. Corticosterone had a diurnal rhythm which had the same peak and nadir as Per1 gene expression in liver and adipose. However, Per 1 expression was not altered by obesity in either tissue.

In adipose tissue, 11 β -HSD1 was markedly decreased in obese mice at both the HPA peak and nadir. There was no difference in the expression of 5 α -reductase or 5 β -reductase. 11 β -HSD2 was identified in adipose tissues and was increased in obese mice at the HPA axis peak. Interestingly, there was also a decrease in the glucocorticoid receptor (GR) expression.

In liver from obese mice, 11 β -HSD1 was decreased at the nadir but not the peak of the HPA axis. This eliminated the diurnal rhythm of 11 β -HSD1 seen in liver from lean mice. There was also a decrease in 5 α -reductase at both peak and nadir. In addition, a decrease in the GR was observed.

Diet-induced obesity results in increased circulating POMC and corticosterone. In adipose tissue from the obese mice, the decreased 11 β -HSD1 and increased 11 β -HSD2 expression (at the HPA peak) would reduce corticosterone levels. However perhaps more importantly, the marked decrease in GR would reduce glucocorticoid action. In the livers from obese mice, the changes in the genes favored more corticosterone production but the marked decrease in GR would reduce glucocorticoid action. Therefore, in this obesity model, the upregulated HPA axis is accompanied by changes in genes regulating corticosterone regeneration which could lead to decreased glucocorticoid action in liver and adipose tissues.

Sources of Research Support: AstraZeneca, Alderley Park, UK and the NIHR, Manchester Biomedical Research Centre.

Nothing to Disclose: EH, AY, AVT, BL, AW

Pub # P2-463

Session Information POSTER SESSION: CLINICAL - Lipids & Obesity Case Reports (1:30 PM-3:30 PM)

Title Quetiapine-Associated Dyslipidemia and Hyperglycemia in a Pediatric Patient with Overweight

Author String E Carreno, V Dasari, A Badheka, S Yogananda, S Dave-Sharma
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Body

Background:
Medications such as atypical antipsychotic like Quetiapine can cause undesirable alteration in lipid profile and hyperglycemia.

Case Report:
A 15 yr old overweight Hispanic asymptomatic boy referred to the endocrine clinic by his primary care provider due to high serum cholesterol, triglyceride and glucose levels. No personal or family history of hypercholesterolemia, early cardiovascular event or diabetes. Past history was significant for Autism, Anxiety with depression and Attention Deficit Disorder for which he was getting Psychiatric care and was Quetiapine 50 mg daily for >2yrs. Physical examination was significant for increased adiposity and BMI in the overweight range (85-95th percentile) with pubertal tanner staging 5. Levels at the time of presentation showed elevated fasting cholesterol level of 388 mg/dL, triglyceride (TGs) 1420 mg/dL, HDL 46 mg/dL, (LDL could not be calculated due to abnormally high TGs) and glucose 120 mg/dL. Repeat levels of Lipid panel and glucose showed similar results with elevated TGs, cholesterol and glucose 2 days later. LFTs showed elevated AST 47 u/dL and ALT 105 u/dL. Amylase, lipase, thyroid stimulating hormone, fasting insulin and urine micro albumin levels were normal. Electrocardiogram, echocardiogram and cardiac examination was normal. Quetiapine was discontinued immediately and replaced by Aripiprazole by psychiatrist. Lifestyle changes including dietary and exercise program was started. The laboratory tests repeated 4 months later showed normalizing serum cholesterol 209 mg/dL, triglycerides 261 mg/dL, HDL 44 mg/dL, LDL 113 mg/dL, AST 46 u/dL and ALT 68 u/dL with normal Amylase, lipase, thyroid stimulating hormone and fasting insulin levels.

Conclusion:
In past decades, the use of 2nd generation and atypical anti-psychotic is increasing in young population ¹. The cardiometabolic adverse effects and tolerability is well known in adult population ² but in pediatric population, cardiometabolic adverse effects can be pronounced specially with long term use (>24-36 months), obesity, use of multiple psychotropic medications, family history of coronary artery disease and diabetes³. Pediatricians should closely follow this patient for their high risk of developing dyslipidemia and hyperglycemia. Routine laboratory studies including fasting lipid profile and glucose should be performed frequently.

1. Olfson M, Blanco C, Liu L, Moreno C, Laje G. National trends in the outpatient treatment of children and adolescents with antipsychotic drugs. *Arch Gen Psychiatry*. 2006;63(6):679-685

2. McIntyre RS, Konarski JZ. Tolerability profiles of atypical antipsychotics in the treatment of bipolar disorder. *J Clin Psychiatry*. 2005;66(suppl 3):28-36

3. Roger S. McIntyre, MD, FRCPC; Jeanette M. Jerrell, PhD Metabolic and Cardiovascular Adverse Events Associated With Antipsychotic Treatment in Children and Adolescents. *Arch Pediatr Adolesc Med*. 2008;162(10):929-935

Nothing to Disclose: EC, VD, AB, SY, SD-S

Pub # P2-464

Session Information POSTER SESSION: CLINICAL - Lipids & Obesity Case Reports (1:30 PM-3:30 PM)

Title Familial Hypercholesterolemia in African-Americans

Author String HE East, D Riche, C Friedrich, CA Koch
University of Mississippi Medical Center, Jackson, MS

Body Homozygous familial hypercholesterolemia (HFH) is a rare disorder with a prevalence around one in one million. Estimations of incidence in AA are lower. We report 3 cases of AA pts with HFH. The first case is an AA female dx by a dermatologist with HFH at age 3 (event free history up to age 24 briefly reported in the 12/09 issue of Pharmacotherapy). Both parents had severely elevated cholesterol levels. She is obese with numerous xanthomas on her elbows, neck, back, and hands, and thickened Achilles tendons. Her total cholesterol = 1011 mg/dL with an LDL= 965 mg/dL upon initial visit at our institution at age 20. Tx consisted of a BAS, cholesterol absorption inhibitor, niacin, statin and intermittent (due to compliance) LDL apheresis, lowering her LDL levels to the upper 100's. She was completely asymptomatic until age 25 when she began experiencing DOE with exertional chest discomfort relieved by rest. Coronary angiography revealed severe triple vessel atherosclerotic cardiovascular disease (ASCVD) and supravalvular stenosis. She underwent triple CABG with ascending aortic repair and patch graft. Her post-operative course was complicated by xanthomatous pressure ulcers. The second case is an AA male diagnosed with HFH at age 12. His total cholesterol =593 mg/dL with an LDL =512 mg/dL on intermittent therapy. He has a FH of elevated cholesterol, but no known ASCVD FH. He has bilateral corneal arcus, elbow xanthomas, upper back and flank, thickened Achilles and extensor tendon xanthomas. Physical findings improved with apheresis. Despite non-compliance, he remains ASCVD free at age 23. It is unusual for pts with uncontrolled FHH to have no clinical evidence of ASCVD by his age, as evidenced by the next pt. The third case is an AA male who was not screened for FHH prior to adolescence despite both parents with known heterozygous FHH. At age 16, he was admitted to the hospital for persistent chest pain and dx with an MI. He developed severe left ventricular dysfunction which interfered with his daily activities. He has numerous xanthomas on the elbow, back, and flank. Excessive non-compliance with appointments had caused the patient to be lost to follow-up. This most severe genetic lipid condition is exceedingly rare in all patients and more so in AA, making the cases of this population quite significant. Pictures of tendonous Achilles xanthomas and tuberous hand xanthomas, as well as images from one of the patient's coronary angiographies will be presented.

Nothing to Disclose: HEE, DR, CF, CAK

Pub # P2-465
Session Information POSTER SESSION: CLINICAL - Lipids & Obesity Case Reports (1:30 PM-3:30 PM)
Title Fatal Statin-Induced Rhabdomyolysis in the Australian Indigenous Population and Vitamin D Replacement -- New Concepts

Author String G Gerrard
Cairns Base Hospital, Cairns, Australia; Launceston General, Launceston, Australia

Body We report three indigenous patients with a long standing history of type 2 diabetes and dyslipidemia treated with atorvastatin for a minimum of 3 years, who presented with biopsy proven Rhabdomyolysis related to statin use. All of them were Vitamin D deficient, two died of respiratory failure attributed to myositis related respiratory muscle atrophy. The third one responded to high doses of vitamin D replacement.

Case 1:

A 61 year old Indigenous gentleman was referred to our hospital with unintentional weight loss of about 20 kg over two months, associated with an unsteady gait.

He had a long standing history of type 2 diabetes mellitus complicated by hypertension and dyslipidaemia for which he was treated with atorvastatin 40mg daily for a minimum of three years.

His biochemistry was noted for CK 36,800 U/L (46 -171), Vitamin D2 insufficient 58 nmol/L. muscle biopsy was consistent with necrotising myositis.

In the ward his respiratory function deteriorated and he developed type 2 respiratory failure with PaCO2 102mmHg which was the cause of his demise.

Case 2:

A 62 year old Indigenous female presented with an unstable gait. She was known to have history of Type 2 diabetes mellitus treated with gliclazide 80mg tds and metformin 500mg tds. Her dyslipidemia initially treated with atorvastatin 20 mg for three years. Her biochemistry was noted for CK 9,000 U/L (46 -171) with 25 hydroxyVitamin D2 was 19 nmol/L (50-150) with normal calcium and parathyroid hormone levels.

Muscle biopsies demonstrated necrotising myositis classical in appearance of statin toxicity. Shortly afterwards she was admitted to intensive care with a hypercapnic respiratory arrest with a pCO2 of 80mmHg and required intubation followed by non invasive ventilation. She eventually died of respiratory failure.

Third case was 47 year old lady with type 2 Dm and hypertension who was on combination of atorvastatin 80 mg daily and gemfibrozil 600mg BD who presented with acute renal failure and dark urine. Plasma CK 276000 U/L and 25 hydroxy vit D2 22 nmol/L and 1,25 dihydroxy vit D3 40 pmol/L, her vasculitic and connective tissue screen came back negative and muscle biopsy confirmed necrotising myositis.

She required haemodialysis and was replaced with high doses of both calcitriol and calcidiol till she achieved 25 hydroxy vitamin D2 levels of 80 nmol/L.

Her CK normalized within 4 weeks.

1. Ballantyne CM, Corsini A, Davidson MH, et al. Risk for myopathy with Statin Arch Intern Med 2003; 163:553-564

2- Statin safety: a systematic review : Malcolm Law, MD, Alicja R. Rudnicka, PhD. Am J Cardiol 2006;97 [suppl]:52C-60C

3- Schachter M. Chemical, pharmacokinetic and pharmacodynamic properties of statins: an update. Fundam Clin Pharmacol 2005; 19:117-125

4- Vitamin D deficiency association with reversible statin induced myositis Ahmed et al translational research 2009 Volume 153 number 1

5- ACC/AHA/NHLBI Clinical Advisory on Statin ACC/AHA/NHLBI Clinical Advisory on the Use and Safety of Statins. Journal of the American College of Cardiology [copy] 2002 by the American College of Cardiology Foundation and the American Heart Association Published by Elsevier Science Inc Vol. 40, No. 3 2002 ISSN 0735-1097/02/. PII S0735-1097(02)02030-2

Nothing to Disclose: GG

Pub # P2-466

Session Information POSTER SESSION: CLINICAL - Lipids & Obesity Case Reports (1:30 PM-3:30 PM)

Title First Case Report of Acquired Generalized Lipodystrophy Associated with Hypogammaglobulinemia

Author String B Halpern, M Nery, CE Rochitte, TC Martins, MT Barros, MAA Pereira
Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil; Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil; Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil

Body **Introduction:** Acquired generalized lipodystrophy (AGL) is a very rare disorder characterized by postnatal loss of adipose tissue from significant parts of the body, generally during childhood and adolescence(1). It usually presents with diabetes mellitus, due to marked insulin resistance, associated with hypertriglyceridemia and hepatomegaly, but there usually is considerable clinical heterogeneity. AGL can either present after an episode of panniculitis (type 1), in association with autoimmune diseases (type 2) or idiopathically (type 3)(1). In some patients, hypergammaglobulinemia associated with low C4 levels might also be present (2). Besides that, the pathogenesis is not well comprehended. We herein describe a case of AGL in a patient with common variable immunodeficiency (CVID), a condition known for its high rate of recurrent pyogenic infections and associated autoimmune diseases. **Case Report:** A 24-year-old man was referred to us with a two-year history of poorly controlled diabetes mellitus associated with severe hypertriglyceridemia and hepatomegaly. At first glance, an obvious pattern of fat loss on the face, trunks and limbs was noticed. Photographic analysis of three years earlier showed that the patient seemed to be slightly obese with no lipodystrophic signs. His past medical history was noteworthy for two hospitalizations during childhood, one due to a severe pneumonia and the other to an unexplained anemia, which resolved spontaneously. He denied any episode of panniculitis. Biochemical evaluation revealed elevated aminotransferases, primary hypothyroidism with negative thyroid antibodies and marked hypogammaglobulinemia (IgA, IgG and IgM). A diagnosis of CVID was made after exclusion of secondary causes. Ultrasound examination was suggestive of autoimmune thyroiditis and confirmed the hepatomegaly. A total-body RMI confirmed generalized loss of fat. **Conclusion:** CVID is a condition highly associated with autoimmune diseases (3), which commonly presents with negative serum autoantibodies. On the other side, AGL is a poorly understood disease, with the majority of cases presumably related to an autoimmune reaction against adipose tissue. To our knowledge, this is the first case report of AGL in a patient with CVID, who also presents autoimmune thyroiditis. These findings strongly support the role of an autoimmune reaction in the pathogenesis of fat loss in this patient and can be helpful to the better understanding of AGL.

(1) Misra A, Garg, A., Medicine (Baltimore) 2003; 82: 129-46

(2) Savage DB et al., JCEM 2009; 94 (1):10-16

(3)Cunningham-Rundles C, J Clin Immun 2008; 28 S1: S42-S45

Nothing to Disclose: BH, MN, CER, TCM, MTB, MAAP

Pub # P2-467

Session Information POSTER SESSION: CLINICAL - Lipids & Obesity Case Reports (1:30 PM-3:30 PM)

Title The Use of Simvastatin and Cholestyramine: Lessons from Treating 6 Children with Severe Hypercholesterolemia

Author String N Lek, F Yap
KK Women's and Children's Hospital, Singapore

Body **Background:** Hypercholesterolemia (HC) leads to atherosclerotic changes beginning in childhood. Meta-analyses reported that lipid-lowering medications can reduce cholesterol (C) by one-third, but this degree of reduction is inadequate to normalize C in children with severe HC.

Clinical cases: Six non-obese (BMI SDS -2.9 to +0.5) non-diabetic children presented with xanthoma and one or more first-degree relative with HC. Baseline total-C (TC) and LDL-C were markedly raised with normal HDL-C (1.0 to 1.6mmol/l) and triglycerides (0.6 to 1.9mmol/l). Length of follow-up ranged from 0.7 to 6.6 yrs. The patients (P1 to P6) received simvastatin and/or cholestyramine in addition to diet and lifestyle modification.

At 2.5 yrs, P1 had TC 17.9mmol/l, LDL-C 16.0mmol/l. She was started on cholestyramine due to young age. LDL-C dropped by 34% and levelled out at 10.5. P2 presented at 5.6 yrs; TC 18.8, LDL-C 17.4. After 6 months of cholestyramine, LDL-C dropped to 10.4 (40% reduction). These two cases illustrated that cholestyramine alone is inadequate.

P3 presented at 9.9 yrs; TC 8.7, LDL-C 6.9. After 6 months of cholestyramine, LDL-C dropped to 4.7. Simvastatin was added and LDL-C levelled out at 3.5 (49% reduction). He then chose to stop cholestyramine. For the next 5.5 yrs, LDL-C was between 3.0 and 4.5 (median 3.7) with simvastatin only. Creatinine kinase and liver enzymes (ALT and AST) remained normal.

P4 presented at 10.7 yrs; TC 9.1, LDL-C 7.3. After 6 months of simvastatin, LDL-C was unchanged and cholestyramine was added. Within 6 months, the combination normalized LDL-C to 2.1. Then simvastatin had to be stopped because ALT and AST were raised. For the next 4.5 yrs, LDL-C remained stable between 2.7 and 4.9 (median 2.8) with cholestyramine only.

P5 and P6 are two sisters who presented at 7.2 yrs (TC 10.3, LDL-C 8.5) and 8.5 yrs (TC 14.0, LDL-C 12.4). Building on our experience with P4, both were started on simvastatin and LDL-C dropped by 35% and 39%. The addition of cholestyramine then normalized LDL-C to 1.8 (79% reduction) and 1.6 (87% reduction).

Conclusion: Our case series demonstrated that it is possible to normalize C in children with HC by priming with simvastatin and then adding cholestyramine. Simvastatin may subsequently be stopped to minimize the possibility of side-effects. Given the high prevalence of childhood obesity and the importance of treating HC in symptomatic children with family history, a clinical trial is warranted to confirm our observation.

Nothing to Disclose: NL, FY

Pub # P2-468

Session Information POSTER SESSION: CLINICAL - Lipids & Obesity Case Reports (1:30 PM-3:30 PM)

Title Successful Reversal of Severe Hypertriglyceridemia with Insulin Administration in a Non-Diabetic Patient with Alcohol-Induced Acute Pancreatitis

Author String A Omer, A Rathod, D Harlan, J Mordes, MC Blendea
University of Massachusetts, Worcester, MA

Body Background: Severe hypertriglyceridemia (>1000 mg/dL) is a relatively rare but potentially life threatening condition in patients with acute pancreatitis. It requires intervention, but the optimal treatment in the acute care setting has not been well defined. Insulin infusion has been recommended for diabetic patients with severe hypertriglyceridemia, but it is not known if it can be safely administered to normoglycemic patients. Clinical Case: A 46 year old male with past medical history of alcohol abuse and mild fasting hypertriglyceridemia (262 mg/dL; normal <150 mg/dL) presented to the Emergency Department with abdominal pain, and vomiting after significant alcohol intake. He was diagnosed with acute pancreatitis, which was confirmed by abdominal CT scan and an elevated serum lipase concentration. His serum lipoprotein profile revealed a total cholesterol of 705 mg/dL (n: < 200 mg/dL), triglycerides > 10,450 mg/dl on 3 occasions, and a HDL cholesterol of 22 mg/dL (40-59 mg/dL). His liver function tests showed mild elevation of total bilirubin (1.34 mg/dl, normal 0.3-1.2 mg/dL), ALT (147 IU/L, normal 10-40 IU/L) and AST (202 IU/L, normal 10-40 IU/L) and non fasting blood glucose of 96 mg/dL. His serum triglyceride level was unchanged after 36 hr of fasting. Fibrates were not administered due to his elevated liver enzymes. We elected to treat the patient in the Intensive Care Unit to achieve a triglyceride goal < 1000 mg/dL by infusing regular insulin (3 units/hr) and 5% dextrose (100 ml/hr). The infusion was completed after 18 hrs, and his serum triglyceride level decreased to 1044 mg/dL within 24 hr of starting the insulin infusion. Two instances of asymptomatic borderline hypoglycemia occurred 8 and 14 hrs after the insulin infusion was started. The first low blood glucose (57 mg/dL) was treated with a 50 ml intravenous infusion of 50% dextrose, and the second low blood glucose of 75 mg/dL was treated with 50 ml 50% dextrose followed by 10% dextrose (100 ml/hr) and reduction of the insulin infusion rate to 2 units/hr. The patient was discharged home with a serum triglycerides of 815 mg/dL on a regimen of gemfibrozil, low dose aspirin, simvastatin and fish oil. Clinical lesson: Acute pancreatitis associated with severe hypertriglyceridemia in a non-diabetic patient can be treated safely in the ICU with an insulin/dextrose infusion to lower the triglyceride level.

Nothing to Disclose: AO, AR, DH, JM, MCB

Pub # P2-469

Session Information POSTER SESSION: CLINICAL - Lipids & Obesity Case Reports (1:30 PM-3:30 PM)

Title Transient Hypercholesterolemia with Intertriginous Xanthoma in an Infant

Author String E-G Yoo, JH Park
CHA University, Sungnam, Republic of Korea

Body

Background: A variety of genetic conditions are associated with type IIa hypercholesterolemia. The most common form is familial hypercholesterolemia, which is an autosomal codominant disorder. Other rare cause of early hyperbetalipoproteinemia include: Familial defective ApoB100 (FDB), autosomal recessive hypercholesterolemia (ARH), autosomal dominant hypercholesterolemia (ADH). And they are, in most cases, considered as irreversible conditions.

Clinical case: 15-month-old girl presented with yellow dermal plaques in flexural areas. The lesions were first noticed by her parents at 3 mo of age. She was born to healthy unrelated parents, and there was no family history of dyslipidemia or premature coronary disease. She was on breast feeding and home-made weaning diet.

Her total cholesterol was increased at 675 mg/dL, LDL-cholesterol was as high as 540mg/dL, whereas triglyceride and HDL-cholesterol was normal at 51mg/dL, and 46.2mg/dL, respectively. Her lipid profile was compatible with homozygous FH, but the lipid profiles of her parents were normal.

Her physical examination was unremarkable except for xanthomas on wrist, antecubital fossae, neck, and gluteal folds. These findings were consistent with intertriginous xanthoma, a rare subtype of planar xanthoma usually considered specific for homozygous FH. Skin biopsy was performed, and histopathological examination showed many foam-laden histiocytes in the dermis, which was consistent with xanthoma.

The patient was presumptively diagnosed with FH. Although FH poorly responds to medical therapy, low fat diet and bile acid sequestrant therapy (Cholestyramine 0.24g/kg/day) were tried initially. Unexpectedly, the subsequent cholesterol levels decreased rapidly and the xanthomas also began to regress. Cholestyramine was gradually tapered, but her cholesterol level was maintained at normal range even after discontinuation of cholestyramine therapy. Now she is 3 years old, and it is 8 months since cholestyramine was discontinued, and her plasma lipid levels are consistently normal. The xanthomas are still visible, but much thinner than before.

Conclusion:
The significance of this clinical case is noticed by its reversibility. As mentioned above, most of congenital causes of hyperbetalipoproteinemia are considered irreversible and especially so if they're accompanied with excessively high LDL and xanthomas.

Nothing to Disclose: E-GY, JHP

Pub # P2-470

Session Information POSTER SESSION: CLINICAL - Lipids & Obesity Case Reports (1:30 PM-3:30 PM)

Title Severe Complications after Intra-gastric Balloon Insertion in a Patient with Obesity Hypoventilation Syndrome

Author String DF Carvalho, LCS Lima, C Cercato, MC Mancini, M Melo, E Moura, A Halpern
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Body **Background:**The intra-gastric balloon (IGB) is known as a minimally invasive endoscopic therapy for the management of obesity and may be used for preoperative weight loss in obese subjects. Associated renal failure and gastric ulcer are rare, yet dangerous, complications. **Clinical Case:**A 51 y.o. woman was followed in our service for severe obesity (BMI 54.2 kg/m²) associated with severe Obesity Hypoventilation Syndrome (OHS) and Pulmonary Hypertension (PH). The patient became oxygen dependent due to her pulmonary conditions. Besides a low caloric diet intake and an exercise program, she had been treated with several anti-obesity drugs for 2 years without significant weight loss or improvement in cardiorespiratory status. While awaiting for obesity surgery, the breast cancer screening was positive and she underwent a core biopsy that showed an *in situ* ductal carcinoma. The preoperative team recommended weight loss before surgery to reduce patient's cardiovascular and pulmonary risk. We indicated an intra-gastric balloon. The IGB was inserted by upper GI endoscopy and the patient stayed in observation for five days because of her low diet acceptance and some vomits. At the fifth day, she received orientations about the IGB and was discharged. Laboratory tests at discharge: Creatinin 0.90 mg/dL, pH 7.31, Bicarbonate 29.6 mmol/L, BE 1.8 mmol/L. Twelve days later, the patient was admitted at the Emergency Department for syncope, mental confusion and anuria for the last 2 days. Laboratory tests at admission: Creatinin 11.8 mg/dL, pH 7.39, Bicarbonate 51.5 mmol/L, BE 20.9 mmol/L. At this time, the patient was diagnosed with Acute Renal Failure, probably acute tubular necrosis due to renal hypoperfusion. She was then transferred to the ICU, where she received intravenous saline infusion and hemodialysis. After two days, patient presented with melena and serum hemoglobin dropped 4.2 points. The upper GI endoscopy showed a gastric ulcer Forrester III at gastric fundus, despite the balloon was not disrupted and, thus, the IGB was immediately removed. The patient started to eat on the next day, fully recovered renal function and presented no more melena episodes. She was released from the hospital 10 days after her admission. **Conclusion:**Although the reported overall complication rate of the IGB is 2,8%, some can be life-threatening and should be promptly recognized by the medical staff involved in treatment. Moreover, patient[acute]s awareness about complications is crucial.

Nothing to Disclose: DFC, LCSL, CC, MCM, MM, EM, AH

Pub # P2-471

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Beta Cell Biology (1:30 PM-3:30 PM)

Title ARX Contributes to Development and Differentiation of Endocrine Cells in Human Pancreas

Author String M Itoh, S Okazaki, R Miyata, T Inoue, M Hayashi, Y-i Goto
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Body Aristaless-related homeobox gene (ARX) mutation leads to several neurological disorders including X-linked lissencephaly with abnormal genitalia (XLAG), West syndrome and Partington syndrome, with XLAG being the most severe form (1). Although some of the brain pathology of XLAG has already been described, the crucial extra-brain symptoms are severe growth retardation, transient hyperglycemia and intractable diarrhea (1,2). Since ARX expresses in the islets of Langerhans during the embryonic stage, these visceral phenotypes may be related to a loss of ARX function, which develops endocrine cells in the pancreas (3). We investigated the abnormal pancreatic development of XLAG patients with ARX-null mutation. We performed immunohistochemistry of XLAG pancreases, using the antibodies against glucagon, insulin, somatostatin, pancreatic polypeptide, ghrelin, Brn4, Nkx2.2, Mash1, amylase and pancreatic lipase. As the results, the glucagon- and pancreatic polypeptide-producing cells were found to be completely deficient in the islets of Langerhans. We also discovered marked interstitial fibrosis, small exocrine cells with loss of amylase-producing cells and an enlargement of the central lumen of the glandular acini. These pathological findings indicate that ARX contributes to endocrine development, but also to exocrine development of the human pancreas, and its deficiency may lead to the severe phenotypes of XLAG patients.

(1) Kitamura K et al., Nat Genet 2002; 32: 359
(2) Okazaki S et al., Acta Neuropathol 2008; 116: 453
(3) Chen S et al., Nat Chem Biol 2009; 5: 258

Sources of Research Support: Grant of the Ministry of Health, Labor and Welfare in Japan.

Nothing to Disclose: MI, SO, RM, TI, MH, Y-IG

Pub # P2-472

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Beta Cell Biology (1:30 PM-3:30 PM)

Title Effects of Adipocytokines and Free Fatty Acids on Viability and Apoptosis of Rat INS-1E β -Cells

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Body

Background and Aims:
The molecular interactions between adipose tissue and β -cells remain to be elucidated. Obesity is associated with a dysregulation of adipocyte secretory function. We asked, whether the adipocytokines, leptin, adiponectin, Nampt and vaspin, or the free fatty acids palmitate and oleate directly influence INS-1E β -cell survival and function.

Methods:
Changes in viability of INS-1E cells were detected by WST-1 assay. Adenylate kinase release was determined to assess cytotoxicity. Apoptosis was measured by Annexin V/PI Assay. For further characterization of activated apoptosis signaling pathways, Western blotting of p53, caspase-3 and NF- κ B was performed. Insulin secretion was measured by ELISA.

Results:
A dose-dependent increase (98 \pm 6.4%) in insulin secretion was detected after stimulation with 20mM glucose. Viability was significantly decreased by 91 \pm 6.2% after stimulation with IL-1 β [10ng/ml], 46 \pm 7.8% by TNF α [10ng/ml] and 26.2 \pm 6.1% by IFN γ [10ng/ml]. Cytotoxicity was increased by 40% after stimulation with a cytokine combination (IL-1 β +IFN- γ [10ng/ml+10ng/ml]). In contrast, adipocytokines showed no effect on viability and cytotoxicity. Palmitate induced cytotoxicity by 70%, oleate by 40% and a palmitate/oleate combination by 20%. Apoptosis was increased after stimulation with a cytokine combination(IL-1 β +IFN- γ [10ng/ml+10ng/ml]) by 63 \pm 9% and with palmitate[0.5mM] by 20 \pm 3.6%. Oleate[0.5mM] and a palmitate/olea combination[0.25mM+0.25mM] decreased apoptosis by 20 \pm 4%. In contrast and most importantly, adipocytokines showed no effects on apoptosis. In addition, adipocytokines did not protect against cytokine-induced apoptosis. Furthermore, no activation of apoptosis signaling pathways were found after stimulation with adipocytokines.

Conclusion:
According to our results, leptin, adiponectin, Nampt and vaspin do not induce β -cell death, nor do they influence viability and cytotoxicity in the rat β -cell model INS-1E. However, free fatty acids, particularly palmitate, exert direct effects on β -cell survival.

Nothing to Disclose: RS, TG, SS, AG, SL, PK, JK, AB-S, AK, WK

Pub # P2-473

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Beta Cell Biology (1:30 PM-3:30 PM)

Title Small Molecules Targeting cAMP Generation Restore β -Cell Function in Islets from Type 2 Diabetic Patients

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Body Accumulating data suggest that nitric oxid (NO) produced by inducible NOS (iNOS) play an essential role in the β -cell dysfunction and apoptosis. The main purpose of this study was to investigate whether the expression of iNOS in the islets of diabetic subjects could be modulated by small molecules which increase cAMP generation by targeting adenylate cyclase and phosphodiesterase. iNOS expression was analyzed by confocal microscopy, Western blot and qPCR in islets from both human type 2 diabetic and non-diabetic donors. Hormone secretion was determined with RIA. Cell viability was measured with ELISA.

Confocal microscopy revealed that iNOS is expressed in pancreatic islet cells (insulin, glucagon and somatostatin) of type 2 diabetic subjects and that no iNOS was detected in islets from normal human donors. iNOS mRNA and protein expression was markedly higher in type 2 diabetic islets vs normal human islets. The hormone secretory pattern of type 2 diabetic pancreatic islets incubated at different glucose concentrations showed a reduced insulin secretion response to glucose. Cell viability assay showed that islet cells of type 2 diabetic subjects are more sensitive to long-term culture in glucotoxic environment. Islets incubated with PDE inhibitors IBMX or Rolipram showed an increased insulin secretion while islets incubated with Zaprinast displayed a less increase then islets incubated with Rolipram or IBMX. Our data shows that long term hyperglycaemia seen in human type 2 diabetic patients, based on their HbA1c, might result in the expression of iNOS and thus influencing b-cell viability in the pancreatic islets consequently could disrupt the normal b-cell function. Our data also shows that small molecules targeting adenylate cyclase and phosphodiesterase could be important players to restore β -cell function in type 2 diabetic patients.

Nothing to Disclose: AB, SM, RK, AS

Pub # P2-474

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Beta Cell Biology (1:30 PM-3:30 PM)

Title Poly(ADP-Ribose) Polymerase 1 Deficient Mice Are Protected from Streptozotocin-Induced Diabetes but No Caerulein-Induced Pancreatitis, without a Clear Association with Changes in Reg Protein Expression

Author String B Li, C Luo, J-L Liu
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Body In response to DNA damage, poly(ADP-ribose) polymerase 1 (Parp1) is a key mediator of apoptotic and necrotic cell death. Parp1 deficient mice are resistant to streptozotocin-induced β -cell death and diabetes and Parp1 inhibition or gene deletion attenuates the severity of acute pancreatitis. Reg family proteins have been implicated in cell proliferation, survival and regeneration of endocrine and acinar pancreas. Parp1 inhibitor nicotinamide after 90% pancreatectomy, experimental diabetes and acute pancreatitis all induce Reg gene expression in the pancreas; Parp1 also activates Reg1 promoter and gene transcription. We propose Reg proteins are associated with Parp1 inhibition and the protection of pancreatic islet or acinar cells. This study was designed to test whether the protection provided by Parp1 gene deficiency was connected with specific induction of Reg family genes. Parp1 deficient and wild-type mice were injected with streptozotocin to induce diabetes. Wild-type mice exhibited rapid onset, severe hyperglycemia from 4 to 7 d with blood glucose level exceeding 500 mg/dL. As reported, Parp1 deficient mice displayed normoglycemia upto 4 d and slight hyperglycemia of 274 mg/dL at 7 d; serum insulin level was maintained at 2-fold higher than that of the wild-type mice, confirming a clear protection against diabetes. Seven days after streptozotocin, we measured changes in mRNA, protein levels and immunohistochemistry and showed similar levels of induction of Reg2, -3 α and -3 β genes in the pancreas of wild-type as well as Parp1 deficient mice. We next induced acute pancreatitis in Parp1 knockout and wild-type mice with 7 hourly injection of caerulein. Eleven hours after the initial injection, serum amylase level was increased 4-fold compared to untreated mice, histological HE staining showed neutrophil infiltration and necrosis of acinar pancreas. Surprisingly, unlike being reported, Parp1 knockout caused no effect on the severity of pancreatitis. Likewise, the up regulation of pancreatic Reg2 and -3 β genes upon caerulein treatment, measured at mRNA, protein and immunohistochemistry, was at similar scales in Parp1 deficient and wild-type mice. Our results confirmed the protection of Parp1 gene deletion on islet β -cells but questioned its effect on acinar cells in acute pancreatitis. Protection or not, the significant induction of Reg family genes during experimental diabetes and pancreatitis seemed independent or upstream of Parp1-mediated cell death.

Sources of Research Support: Canadian Institutes of Health Research; National Science and Engineering Research Council of Canada;China Scholarship Council.

Nothing to Disclose: BL, CL, J-LL

Pub # P2-475

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Beta Cell Biology (1:30 PM-3:30 PM)

Title β -Cell Protective Effect of 2-Aminobicyclo[2.2.1]heptan-2-Carboxylic Acid (BCH) as Glutamate Dehydrogenase (GDH) Activator in *db/db* Mice

Author String SJ Han, S-E Choi, HJ Kim, KW Lee, Y Kang
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Body **Aim:** Glutamate dehydrogenase (GDH) is mitochondria enzyme that converts glutamate to α -ketoglutarate, and vice versa, playing an important role in amplification of glucose-stimulated insulin secretion (GSIS). Recently we found that GDH activator, 2-aminobicyclo-heptan-2-carboxylic acid (BCH) had a strong protective effect on the high glucose/palmitate (HG/PA)-induced INS-1 cell death. We initially examined whether BCH restored HG-induced reduction of GSIS, and HG/PA-induced reduction of insulin gene expression. Next, we investigated which death-related signal is affected by BCH. Finally, we studied the effects of BCH on glycemic control and pancreatic β -cell integrity in *db/db* mice.

Methods: INS-1 cells were incubated with 25 mM glucose and 0.5 mM palmitate in the absence or presence of 10 mM BCH. We evaluated GSIS, insulin gene expression, and DNA fragmentation. An *in vivo* study was performed, in which seven-week-old diabetic *db/db* mice were treated with BCH (0.7g/kg, n = 10) and with placebo (n = 10) for 6 weeks. After treatment, an intraperitoneal glucose tolerance test and immunohistologic examinations were performed.

Results: Treatment with BCH restored the HG-induced GSIS inhibition and down-regulated insulin gene expression by HG/PA in INS-1 cells. In addition, treatment with BCH significantly decreased HG/PA-induced INS-1 cell death and phosphor-JNK expression. BCH treatment improved glucose tolerance as well as increasing insulin secretion in *db/db* mice. BCH treatment increased the percentage of insulin-positive β -cell-to-total islet area ($p < 0.05$) and decreased the percentage of β -cell expressing cleaved caspase 3 ($p < 0.05$).

Conclusion: BCH as the activator of GDH improved the glycemic control. This anti-diabetic effect may be associated with improved insulin secretion, and the preservation of islet architecture, and decreased β -cell apoptosis.

Sources of Research Support: New faculty research fund of Ajou University School of Medicine; the Seisun Research Fund.

Nothing to Disclose: SJH, S-EC, HJK, KWL, YK

Pub # P2-476

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Beta Cell Biology (1:30 PM-3:30 PM)

Title Examining the Role of Connective Tissue Growth Factor (CTGF) in β -Cell Replication and Islet Transplantation

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Body CTGF (connective tissue growth factor) is a secreted protein that modulates TGF- β , BMP, and Wnt signaling. In the pancreas, CTGF is expressed in embryonic β cells, ductal epithelium, and pancreatic vasculature but is silenced in β cells, after birth. In other cell types, CTGF plays a role in cell migration and cell proliferation, as well as vasculogenesis. We previously showed that CTGF null mutant mouse embryos have increased a cell mass and a concomitant reduction in β cell mass due to decreased β cell proliferation compared to wild-type embryos. CTGF is re-expressed in maternal β cells during pregnancy suggesting that it plays a role in β cell proliferation under physiologically stimulatory conditions. We hypothesize that CTGF over-expression in adult mice will increase proliferation and subsequently β cell mass. To test this hypothesis, we generated mice in which CTGF over-expression in β cells is doxycycline-dependent. CTGF expression was induced with doxycycline for various time periods (1-10 weeks) to test whether β cell replication or function is enhanced. At earlier time points, there is improved glucose tolerance with CTGF over-expression as compared to later time points. Currently, samples of pancreata are being examined histologically for the effect of CTGF over-expression on β cell proliferation and mass and islet vascularization. In addition, CTGF has been induced in the setting of islet transplantation to determine whether this factor would be beneficial for β cell survival and islet revascularization. Preliminary data suggests that inducing CTGF expression is beneficial to transplanted islets.

Sources of Research Support: JDRF Grant 12007548; NRSA Training Grant 5T32DK706136.

Nothing to Disclose: UG, MG

Pub # P2-477

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Beta Cell Biology (1:30 PM-3:30 PM)

Title Extracellular Ca²⁺ and Protein Kinases Play Important Roles in Mediating *Gymnema sylvestre*-Induced Insulin Secretion from Mouse and Human Islets

Author String A Al-Romaiyan, B Liu, G-C Huang, S Amiel, S Persaud, P Jones
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Body **Background:** plant extracts such as *Gymnema sylvestre* (GS) have been used to treat diabetes mellitus for many centuries. We have shown previously that OSA has a direct effect on insulin secretion but the mode of action has not been studied in detail.
Aim and Methods: We have used isolated mouse and human islets to investigate whether the stimulatory effect of a novel GS extract termed OSA (U.S. Patents 6949261 and 6946151) is dependent on the presence of extracellular calcium or protein kinase activation.
Results: OSA stimulated insulin secretion from mouse and human islets at a substimulatory glucose concentration (2mM) and this was partially inhibited by nifedipine, a voltage-operated Ca²⁺ channel (VOCC) blocker (mouse: 2mM glucose + 0.25mg/ml OSA: 575±71[permil] basal; + 10[μ]M nifedipine: 325±45 [permil]; human: 2mM glucose + 0.25mg/ml OSA: 665±32[permil] basal; + 10[μ]M nifedipine: 370±9 [permil], P<0.001, n=4). OSA stimulated insulin secretion at least, in part, through protein kinase activation since incubating mouse or human islets with staurosporine, a general protein kinase inhibitor, resulted in partial inhibition of OSA-induced insulin secretion (mouse: 2mM glucose + 0.25mg/ml OSA: 650±86[permil] basal; + 200nM staurosporine: 369±69[permil]; human: 2mM glucose + 0.25mg/ml OSA: 673±27[permil] basal; + 200nM staurosporine: 485±19[permil], P<0.0001, n=4). OSA-induced insulin secretion was not mediated by Ca²⁺-sensitive protein kinases but surprisingly was associated with a decrease in intracellular cAMP levels.
Conclusions: These data indicate that the GS isolate OSA stimulates insulin secretion from mouse and human islets *in vitro*, at least in part as a consequence of Ca²⁺ influx and protein kinase activation.

Nothing to Disclose: AA-R, BL, G-CH, SA, SP, PJ

Pub # P2-478

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Beta Cell Biology (1:30 PM-3:30 PM)

Title Effects on Pancreas Alpha and Beta Cell Composition and Glutamate Decarboxylase Expression Due to Lack of Functional GABAB Receptors

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Body We have previously shown that GABA participates, through GABAB receptors (GABABRs), in the regulation of glucose homeostasis. Mice with a deletion of GABABRs (GABAB1KO), present a prediabetic condition, with insulin resistance and high islet insulin content (1). Since GABABRs have been demonstrated to regulate cell proliferation in the central nervous system (2), here we studied islet cell composition (alpha and beta cells) in GABAB1KO and wild-type (WT) adult mice by immunohistochemistry. In addition, we have shown that the absence of functional GABABRs induced an increased expression of the GABA synthesis rate limiting enzyme in the hypothalamus, glutamate decarboxylase (GAD) (3). Here we determined GAD expression by immunohistochemistry in pancreas of adult WT and GABAB1KO mice of both sexes. Islet number per pancreas area did not differ between sexes or genotypes (ANOVA: ns). Islet area was significantly larger in males (M) than in females (F), without differences due to genotype [islet area (μm^2): WT M: 8632.9 ± 1422.1 , GABAB1KO M: 6889.4 ± 1209.7 , WT F: 5354.3 ± 681.3 , GABAB1KO F: 4532.5 ± 574.3 ; two-way ANOVA: interaction: ns, sex: $p < 0.01$, genotype: ns]. Average alpha cell area per islet area was significantly decreased in GABAB1KO mice with regards to WTs and was larger in females than in males [alpha area / islet area (%): WT M: 19.0 ± 1.6 , GABAB1KO M: 15.9 ± 0.8 , WT F: 25.6 ± 3.3 , GABAB1KO F: 18.7 ± 0.9 ; two-way ANOVA: interaction: ns, sex: $p < 0.02$, genotype: $p < 0.02$]. Average beta cell area per islet increased in GABAB1KO and was larger in males with regard to females [beta area / islet area (%): WT M: 81.05 ± 1.6 , GABAB1KO M: 84.13 ± 0.8 , WT F: 74.35 ± 3.3 , GABAB1KO F: 81.24 ± 0.9 ; two-way ANOVA: interaction: ns, sex: $p < 0.02$, genotype: $p < 0.02$]. GAD positive area per islet was increased in GABAB1KO islets in relation to WT, and was larger in males than in females [GAD area/islet (%): WT M: 35.6 ± 2.2 , GABAB1KO M: 39.0 ± 1.2 , WT F: 29.3 ± 2.5 , GABAB1KO F: 35.0 ± 1.5 ; two-way ANOVA: interaction: ns, genotype: $p < 0.04$, sex: $p < 0.02$]. We conclude that the absence of functional GABABRs alters the islet's cell composition, with an increase of beta cell area at the expense of alpha cell area in GABAB1KO mice. In addition, an increase in GAD expression was observed in GABAB1KO mice, in agreement with what was observed previously in the hypothalamus of these mice. These changes may contribute to the alterations in glucose homeostasis regulation observed in GABAB1KO mice.

(1) Bonaventura MM et al., Am J Physiol Endocrinol Metab; 294:E157

(2) Magnaghi V et al., Eur J Neurosci 19:2641

(3) Catalano PN et al., Am J Physiol Endocrinol Metab 298:E683

Sources of Research Support: Grants from Concejo Nacional de Investigaciones Cientificas y Tecnicas (CONICET PIP 363 2010); Agencia Nacional de Promocion Cientifica y Tecnologica (ANPCyT BID PICT 2006 N[ordm]00200 and BID PICT 2007 N[ordm] 01050); Universidad de Buenos Aires (ME 038).

Nothing to Disclose: MC, MMB, BB, CL, VL-L

Pub # P2-479

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Beta Cell Biology (1:30 PM-3:30 PM)

Title The Glial Glutamate Transporter 1 (GLT1) Is Expressed by Pancreatic β -Cells and Prevents Glutamate-Induced β -Cell Death

Author String ES Di Cairano, AM Davalli, L Perego, S Sala, F Sacchi, S La Rosa, G Finzi, C Placidi, C Capella, P Conti, VE Centonze, F Casiraghi, F Bertuzzi, F Folli, C Perego
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Body Glutamate (GLUT) is the major excitatory neurotransmitter of the central nervous system and may induce cytotoxicity through activation of glutamate receptors and oxidative stress. Its extracellular concentration is regulated by high affinity glutamate transporters including the glial glutamate transporter GLT-1. GLUT is co secreted with glucagon by the α -cells in islets, acting as a signalling molecule and hormone secretion modulator. We investigated whether GLUT plays a role on islet cells viability and the role of GLT-1 in this process. We used mouse β TC3, α TC1 cell lines and human islets of Langerhans to test whether the effects of an acute and chronic exposure to GLUT exerts a cytotoxic effect in α and β cells. Cells were grown for 24 h and then exposed to either GLUT, dihydrokainate (DHK) a selective GLT-1 inhibitor, Ceftriaxone (CEF) or GLUT receptor inhibitors (APV and CNQX) for 5 days. Viability was measured by the MTT and apoptosis by TUNEL. GLT1 expression and content in β TC3 cells and human islets was tested by RT-PCR, Western Blotting and confocal microscopy. 5 days incubation with 0.05, 0.5 and 5 mM GLUT increased β TC3 apoptosis by in a dose-dependent fashion up to 53% ($p < 0.01$) as compared to only 20% at 5mM in α TC1 cells ($p < 0.05$). GLT1 was expressed in β TC3 cells while no expression was found in α TC1. These data were confirmed by Na-dependent [3 H]-D-aspartate uptake in β TC3 cells, that was inhibited by DHK. GLT-1-shRNA induced down-regulation of 35%, also caused a 2-4 fold increase in apoptosis in β TC3 incubated in 0.5mM GLUT ($p < 0.001$). In contrast, CEF induced 2 fold increased of GLT1 expression and provided dose dependent protection from glutamate-induced toxicity ($p < 0.05$). GLT1 was expressed exclusively in the cell membrane in the β cells in human pancreas, as demonstrated by double-confocal immunocytochemistry with GLT-1 and insulin antibodies. GLT-1 inhibition with DHK inhibited Na-dependent [3 H]-D-aspartate uptake by 60% in human islets. 5 mM GLUT caused a 80% increase and 0.1 mM DHK caused a 60% in human islets' apoptosis and 50% reduction in 16.7mM glucose-stimulated insulin release and 70% increase in the proinsulin release (all $p < 0.05$). Quantitation by electron-, immunoelectron and double confocal microscopy of apoptotic/degenerated confirmed that apoptosis occurred exclusively in β -cells. GLT1 is a new player in glutamate homeostasis in the islet of Langerhans, controlling extracellular glutamate levels and β -cells integrity.

Sources of Research Support: NIH R01-DKO80148.

Nothing to Disclose: ESDC, AMD, LP, SS, FS, SL, GF, CP, CC, PC, VEC, FC, FB, FF, CP

Pub # P2-480

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Beta Cell Biology (1:30 PM-3:30 PM)

Title Beta Cell Dysfunction, Oxidative Stress and S6K1 Activation in Pancreas in the Zucker Obese Rat Model

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University of Missouri School of Medicine, Columbia, MO

Body

Insulin resistance and β -cell dysfunction are leading components in the pathogenesis of type 2 diabetes mellitus. Obesity is linked to activation of mammalian Target of Rapamycin S6K1 (mTOR/S6K1) pathway, which is implicated in cell proliferation. S6K1 is involved in insulin signaling and leads to serine phosphorylation of IRS-1, triggering its proteosomal degradation and interfering with insulin signaling through Akt. This mechanism, along with oxidative stress has been related to insulin resistance in multiple tissues.

We hypothesize that obesity induced S6K1 activation leads to impaired β -cell function and survival through serine phosphorylation and degradation of IRS-1 and IRS-2, in concert with increased oxidative stress. We utilized the Zucker Obese (ZO) rat, which displays severe obesity as well as glucose intolerance. We performed Intravenous Glucose Tolerance Test in 9 week old male ZO (n=4) and Zucker Lean (ZL) rats (n=4). In the pancreas, we assessed NADPH oxidase activity by spectrophotometry, NADPH oxidase subunit Rac-1, IRS-1, Ser⁶³⁶phospho-IRS-1, IRS-2, Ser⁷³¹phospho IRS-2, Akt, Ser⁴⁷³phospho Akt, S6K1 and Thr³⁸⁹phospho-S6K1 by Western Blot. Results are presented as averages \pm SE. Student t test was used for statistical analysis.

The area under the curve (AUC) for glucose was significantly increased in ZO compared to ZL. Indices of insulin resistance, including HOMA-IR and Insulin Resistance Index ($AUC_{glu} \times AUC_{ins}$) were increased in ZO rats ($p < 0.05$). The Insulinogenic Index ($\Delta_{ins}/\Delta_{glu}$) calculated at 15 and 60 minutes was not different between ZO and ZL animals despite significant hyperglycemia in the ZO group, suggesting impaired compensatory insulin secretion.

NADPH oxidase activity was significantly increased in ZO rat pancreas (15.91 ± 2.94 vs 10.57 ± 1.40 mOD/min/mg) and Rac-1 was significantly increased in ZO. Serine phospho-IRS-1 was increased in ZO animals while serine phospho-Akt was reduced ($p > 0.05$). Total IRS-2 was significantly decreased in ZO animals relative to ZL (9.8 ± 0.99 vs 22.8 ± 4.60 arbitrary units). Serine phosphoIRS-2 was increased in ZO animals, and threonine phosphoS6K1 was increased in ZO ($p > 0.05$).

Our findings are suggestive of severe insulin resistance and impaired insulin secretion in a rodent model of obesity and overnutrition in concert with activation of the S6K1 pathway, increased oxidative stress and defective insulin signaling in the pancreas. These defects can potentially affect β -cell function and survival.

Nothing to Disclose: GL, CBK, CM, JRS

Pub # P2-481

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Beta Cell Biology (1:30 PM-3:30 PM)

Title Low-Molecular-Weight Dextran Sulfate Administration Prevents Type 1 Diabetes in Non-Obese Diabetic (NOD) Mice

Author String S Valle, S Ernst, A Garcia-Ocana
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Body Type 1 diabetes (T1D) is an autoimmune disease that results from islet immune infiltration and cellular cytotoxicity leading to selective and progressive destruction of beta cells. The non-obese diabetic (NOD) mouse model that spontaneously develops T1D shares multiple characteristics with the human disease, and can be employed to unravel clinically relevant and important information for the treatment of this disease. Low molecular weight (≤ 5000 Da) dextran sulfate (DS) is a sulfated polysaccharide with pleiotropic actions in the immune system. DS blocks complement, NK cell-mediated cytotoxicity, and the phenotypic and functional maturation of dendritic cells by reducing T cell proliferation and impairing the secretion of pro-inflammatory mediators such as IL-1 β , IL-6 and TNF- α . DS also protects vasculature and tissues from ischemia/reperfusion injury and improves islet xenograft survival. Importantly, DS infusion in humans is well tolerated and leads to increased levels of the islet protective hepatocyte growth factor. Based on this evidence, we wondered whether DS could prevent the development of diabetes in NOD mice. To answer this question, we daily administered either saline (SAL, n=12) or 200 [μ g]/mouse of DS diluted in saline (n=8) intraperitoneally to 10 week-old female NOD.Ltj mice for 12 weeks. We analyzed non-fasting blood glucose levels (BG) weekly, and measured plasma insulin, beta cell homeostasis and islet insulinitis at the end of treatment. Analysis of BG in these mice revealed that only 1 out of 8 (P<0.05) DS-treated NOD mice develop hyperglycemia (BG \geq 250mg/dl) compared with 8 out of 12 SAL-treated NOD mice. On the other hand, plasma insulin levels were increased in DS-treated mice compared with SAL-treated mice. Importantly, the rate of islets with severe insulinitis was highly reduced in DS-treated NOD mice (41 \pm 17%, P<0.05) compared with SAL-treated NOD mice (78 \pm 12%). The diminished islet infiltration in DS-treated NOD mouse islets correlated with decreased beta cell apoptosis (0.27 \pm 0.14%, DS-treated vs. 10.5 \pm 0.1 SAL-treated) and increased beta cell mass (0.9 \pm 0.03 mg, DS-treated vs. 0.1 \pm 0.1 mg, SAL-treated), reaching similar levels to beta cell mass in non-diabetic NOD female mice at 10 weeks of age before DS treatment. These results clearly demonstrate that DS can decrease islet inflammation, protect beta cells and prevent T1D in NOD mice. Dextran sulfate can be a therapeutic tool for the treatment of T1D.

Sources of Research Support: NIH/NIDDK Grant DK067351-06 and ADA Grant 1-10-BS-59 awarded to AG-O.

Nothing to Disclose: SV, SE, AG-O

Pub # P2-482

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Beta Cell Biology (1:30 PM-3:30 PM)

Title ER Oxidoreductase-1-Like β (ERO1 β) Regulates Susceptibility to ER Stress and Is Induced by Insulin Flux in Pancreatic β -Cells

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University of Michigan Medical Center, Ann Arbor, MI

Body Hyperglycemia increases insulin flux through the endoplasmic reticulum (ER) of pancreatic β cells and the unfolded protein response (UPR) pathway is required to enhance insulin processing. PDX1, a key pancreatic transcription factor, regulates insulin along with targets involved in insulin processing and secretion. Here we find that PDX1 is a direct transcriptional regulator of Ero1 β , which maintains the oxidative environment of the ER to facilitate disulfide bond formation. PDX1 deficiency reduced Ero1 β transcript levels in mouse islets and mouse insulinoma (MIN6) cells; moreover, PDX1 occupied the Ero1 β promoter in MIN6 cells. ERO1 β levels were affected by glucose and redox conditions indicating potential roles in adaptation to increased oxidative protein folding load in the β cell ER. Silencing of Ero1 β in MIN6 cells decreased insulin content and impaired glucose stimulated insulin secretion. ERO1 β deficiency resulted in increased susceptibility to ER stress induced apoptosis. These findings demonstrate roles for ERO1 β in maintaining insulin content and regulating cell survival during ER stress.

Sources of Research Support: NIH Grant F30 DK085931 awarded to CK; NIH Grant P01 DK49210 awarded to DAS; NIH Grant R01 DK48280 awarded to PA.

Nothing to Disclose: CK, JY, GR, YW, JL, PA, DAS

Pub # P2-483

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Beta Cell Biology (1:30 PM-3:30 PM)

Title Exendin 4 Has Direct Effects on Signal Transduction Pathways and Gene Expression in Isolated Hepatocytes
In Vitro Independent of the Pancreatic-Type GLP-1 Receptor and cAMP

Author String SL Samson, EV Gonzalez, C Espiritu, P Sathyanarayana, P Saha, M Bajaj, L Chan
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Body We have previously shown that the glucagon like peptide receptor (GLP-1R) agonist, exenatide (exendin 4 or Ex4) decreases gluconeogenic (Glucose-6-phosphatase and PEPCK) and lipogenic (e.g. fatty acid synthase, stearoyl CoA desaturase I) gene expression in the liver. We also have found that Ex4 has direct effects *in vitro* on gluconeogenesis and lipogenic gene expression in isolated primary hepatocytes. In order to determine the mechanism for these direct effects, we have investigated a number of candidate signal transduction molecules. Phosphorylation of Akt, p70 S6 kinase, and AMP-dependent protein kinase (AMPK) is increased by treatment of primary hepatocytes in culture with Ex4. Moreover, AMPK activity also is increased by *in vitro* Ex4 treatment. Although we observe direct effects on gene expression and cell signaling molecules with Ex4 treatment, we cannot detect significant expression of the GLP-1R RNA or protein in primary hepatocytes. There also is no increase cAMP production with Ex4 treatment, although the positive controls of glucagon and forskolin significantly induce cAMP production under the same conditions. Our findings support direct effect of Ex4 on hepatocytes independent of the pancreatic-type GLP-1R and cAMP activation, possibly involving another as yet unidentified receptor and mechanism.

Disclosures: MB: Researcher, Amylin Pharmaceuticals; Speaker Bureau Member, Sanofi-Aventis; Bristol-Myers Squibb. Nothing to Disclose: SLS, EVG, CE, PS, PS, LC

Pub # P2-484

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Beta Cell Biology (1:30 PM-3:30 PM)

Title Effects of Clinically Relevant Doses of Immunosuppressant Drugs on Human Islet Function and Survival

Author String AM Robledo Reyes, G Poffenberger, C Thompson, C Dai, A Powers
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Body One possible mechanism for pancreatic islet graft failure after islet transplantation is toxicity related to the required immunosuppressive drugs. To assess the effects of clinically relevant levels of tacrolimus (TAC) or sirolimus (SIR) on human islet function and survival, we transplanted 500 human islets beneath the kidney capsule of normoglycemic NOD-*scid* *IL2rg*^{null} (NSG) mice and then treated the mice with IP injections of 0.2 mg/kg every other day of SIR, 1 mg/kg/day of TAC, or saline for four weeks (a different islet preparation was used for each drug). The dose needed to obtain a clinically relevant level of these agents was determined by measuring the trough level in blood. Surprisingly, in NSG mice, SIR had a much longer half-life and TAC a shorter half-life than expected based on dosing in humans or in C57BL/6J mice. Fasting human insulin levels were reduced in TAC-treated mice (0.72 ± 0.13 ng/dl, $n = 6$ vs. control 3.42 ± 0.92 ng/dl, $n=4$; $p<0.01$), but not in SIR-treated mice (1.35 ± 0.32 ng/dl, $n = 5$ vs. control 2.64 ± 0.52 ng/dl, $n=5$; $p>0.05$). TAC treatment also impaired glucose/arginine-stimulated human insulin secretion (1.04 ± 0.11 ng/dl, $n=6$ vs. control 4.38 ± 0.76 ng/dl, $n=4$; $p<0.001$), but glucose/arginine-stimulated human insulin secretion was not different in SIR-treated and control mice (2.17 ± 0.50 ng/dl, $n=5$ vs. control 2.60 ± 0.17 ng/dl, $n=5$; $p>0.05$). The human insulin content of islet grafts was similar in TAC-treated and control mice (2309 ± 581 ng/graft $n=5$ vs. control 2096 ± 543 ng/graft, $n=3$; $p>0.05$) and in SIR-treated and control mice (1615 ± 325 ng/graft $n=4$ vs. control 1383 ± 175 ng/graft, $n=4$ $p>0.05$). The morphology of the human islet grafts was similar in SIR-treated, TAC-treated and control mice. Based on the pharmacokinetics of SIR and TAC in these immunosuppressive mice, some prior studies may have used excessive doses or may have not achieved clinically relevant drug levels. These results suggest that a clinically relevant level of TAC but not SIR impairs human beta cell function without affecting beta cell mass.

Nothing to Disclose: AMRR, GP, CT, CD, AP

Pub # P2-485

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Beta Cell Biology (1:30 PM-3:30 PM)

Title Bone Morphogenetic Protein 4 Inhibits Insulin Secretion in Rat Islets

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University of Copenhagen, Copenhagen, Denmark; Karolinska Institutet, Stockholm, Denmark

Body **Aim:** The aim of the present study was to investigate the effects of bone morphogenetic protein 4 (BMP4) on beta-cell function.

Background: BMP4 is essential in the developing pancreas, but little is known about its effects on function of the mature beta-cell. Previously, we have shown that BMP4 is expressed in islets and inhibits beta-cell proliferation under both basal and growth factor-stimulated conditions. This effect can be reversed by incubation with the extracellular domain of the BMP receptor, ALK3. We hypothesized that BMP4 also has an effect on glucose-stimulated insulin secretion (GSIS) from cultured beta-cells.

Methods: GSIS from neonatal rat islets exposed to BMP4 or ALK3 was evaluated by perfusion. Batch incubation of islets was applied to study the temporal effects of BMP4 on insulin secretion after exposure to high or low glucose. Finally, in order to investigate the mechanisms underlying the observed reduction in insulin secretion, we measured membrane capacitance in islets pre-incubated with BMP-4 and control islets using patch clamp technique.

Results: Glucose as well as forskolin-stimulated insulin secretion was inhibited in islets pre-cultured with BMP4 and enhanced in islets pre-cultured with ALK3. The inhibitory effect was time-dependent with maximal inhibition by BMP4 after 4 days of exposure. Islet insulin content was not affected by BMP4 nor was the expression of *Ins1* and *Ins2* mRNA measured by RT-PCR. Furthermore, preliminary data using patch clamp recordings, indicated that membrane capacitance in islets was reduced and calcium currents inhibited significantly by BMP4.

Conclusion: BMP4 inhibits beta-cell function, an effect that was reversed by BMP4 receptor blockade. The inhibitory effects of BMP4 could partly be due to defects in the secretory machinery. Inhibitors of endogenous BMP4 may have clinical potential to de-repress beta-cell function.

Nothing to Disclose: MLBJ, CB, LE, TM-P, NB

Pub # P2-486

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Beta Cell Biology (1:30 PM-3:30 PM)

Title Differential Roles of Insulin and mTOR Signaling in Nutrient-Induced β -Cell Neogenesis in Larval Zebrafish

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Body The pancreatic β -cells have a critical role in regulating glucose homeostasis by secreting insulin on demand. A failure to secrete sufficient insulin to maintain normoglycemia can result in Type 2 diabetes. One mechanism to maintain adequate production of insulin to meet elevated metabolic demand is to increase the number of β -cells through replication or neogenesis. Zebrafish also maintain normoglycemia and provide a unique opportunity to investigate the regulation of β -cell number in real time. To this end, we are using a variety of transgenic and mutant zebrafish lines to investigate the regulation of β -cell number following increased metabolic demand. We have found that feeding 6 day-old larvae a diet composed primarily of protein and fat increased the β -cell number from 32 to 45 within 8 hours. However, the number of BrdU-positive β -cells was not increased, suggesting that the new β -cells resulted from neogenesis rather than replication. This increase was partially blocked by inhibitors of PI3K or MEK1/2, but not by the mTOR inhibitor rapamycin. In addition, treating 6 day-old larvae solely with 20mM glucose increased the number of β -cells from 32 to 38 within 8 hours. However, this response to glucose was blocked by the mTOR inhibitor but not by the inhibitors of PI3K or MEK1/2, suggesting that different mechanisms regulate β -cell neogenesis depending on the type of nutrient stimulation. To investigate the role of insulin/IGF signaling in the regulation of β -cell numbers, we generated transgenic lines that express dominant-negative IGF1R (dnIGF1R). Interestingly, expression of the dnIGF1R in skeletal muscle significantly increases the number of β -cells in 2 week-old fish, suggesting that zebrafish skeletal muscle is an insulin target tissue although an insulin-sensitive glucose transporter has not been identified in teleosts. Intriguingly, expression of the dnIGF1R in the β -cells of 6 day-old larvae completely abolished the rapid β -cell increase induced by the protein and fat diet but the increase induced by glucose was not affected. This suggests a role of β -cells themselves in regulating nutrient induced neogenesis. Studies are ongoing to determine the origin of the new β -cells induced by feeding and peripheral insulin resistance and to investigate the signaling mechanisms that regulate nutrient induced β -cell neogenesis

Nothing to Disclose: LM, WC

Pub # P2-487

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Beta Cell Biology (1:30 PM-3:30 PM)

Title The Antioxidation Effect of Vitamin D in Cultured β -Cells May Be through a Reduction of ROS Activation

Author String VM Bota, F Mehdi, F Xiong, L Luo
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Body Background: Numerous epidemiological studies show a strong correlation between diabetes and Vitamin D (VD). Previously we reported that VD improves insulin secretion, prevents apoptotic changes which may result in cell survivability. Although other studies reported that VD has dose specific anti-oxidation on non β -cell-lines, it can also be critical for the β cell function/survival. In this study, we explored whether VD's anti-oxidation is through reduction in intracellular reactive oxygen species (ROS) in cultured β cells. Methods: A rat pancreatic INS-1 beta cell line obtained from an X-ray induced insulinoma was cultured using RPMI 1640 medium, enriched with glucose, pyruvate, mercaptoethanol, penicillin and streptomycin. We use Streptozotocin (STZ) in various concentrations in order to establish an oxidative stress model on the pancreatic beta cell. The ROS were labeled using reactive oxidant-sensitive fluorescent probe 5-(and-6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate (CM-H2DCFDA) and was measured by flow-cytometry analysis (LSRII). We then investigated whether concomitant treatment with 1,25-dihydroxyvitamin D at physiological concentration is able to restore beta cell oxidation. Results: Administration of 1, 25-dihydroxyvitamin D to β cell culture resulted in reduction of intracellular ROS measured as H2DCFDA positive cell percentage ($9.57\% \pm 1.21$ vs. $18.8\% \pm 0.57$ without VD control; $N=3$, $p<0.05$). In order to establish a cellular oxidation culture model, we tested STZ from a range of 30 μ M to 5 mM. We found that 2 mM is the optimal dosage for induction of β cell oxidation. In this model, concomitant treatment with 1,25-dihydroxyvitamin D at physiological concentration of 10^{-8} M for 3 hours resulted in reduction in the level of intracellular ROS ($13.47\% \pm$ vs. $19.7\% \pm$ STZ only control $N=3$, $p<0.05$). Conclusion: Vitamin D prevents rat pancreatic β cell oxidation in vitro and it may be through a reduction of the ROS activity. More studies are needed to clarify the involved mechanisms. This study may provide information for the potential benefit of VD in diabetic patients. However, clinical investigations are needed for defining the suitable application for patients.

Nothing to Disclose: VMB, FM, FX, LL

Pub # P2-488

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Beta Cell Biology (1:30 PM-3:30 PM)

Title Osteoprotegerin, a Novel Downstream Target of Lactogens, Directly Enhances Human Beta-Cell Survival and Proliferation

Author String N Guthalu Kondegowda, A Otero, A Mozar, C Chin, RC Vasavada
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Body Osteoprotegerin (OPG), a soluble glycoprotein of the tumor necrosis factor receptor superfamily, was originally identified as a key regulator in bone metabolism. Recently, it has been shown that OPG is also expressed in the pancreas; enhances rodent β -cell survival against cytokines; and is significantly increased at the mRNA level in three different models of beta cell expansion, including pregnancy; suggesting a role for OPG in β -cell biology. However, nothing is known about the effects of OPG on human β -cells. Previously we found that lactogens protect β -cells against varied cell death inducers including glucolipototoxicity (GLT). To identify novel downstream targets of lactogens potentially involved in its pro-survival effects, we ran apoptosis gene expression arrays on normal (NL) and placental lactogen expressing transgenic (TG) mouse islets treated with and without GLT. OPG expression was increased in TG islets under control (22-fold) and GLT (9-fold) conditions compared to NL islets. A similar significant increase in OPG expression was seen using real time PCR, confirming these observations. Furthermore, OPG mRNA expression was increased in INS1 cells acutely treated with prolactin under control (6-fold) and GLT (1.5-fold) conditions. These results identify OPG as a novel downstream target of lactogens in β -cells. We next sought to determine if OPG had a direct effect on human β -cell survival. GLT-induced β -cell death in human islet cell cultures (n=3) treated with and without OPG (200ng) was measured by insulin and TUNEL co-staining. GLT caused a 3.5-fold increase in β -cell death which was significantly ameliorated by ~60% with OPG treatment, demonstrating a pro-survival effect of OPG on human β -cells. Based on the increase in OPG mRNA in rodent models of β -cell expansion, we analyzed the effect of OPG on human β -cell proliferation, by insulin- bromodeoxyuridine (BrdU) co-staining of human islet cell cultures (n=2-4) treated with different concentrations of OPG (25-200ng) in the presence of BrdU. There was a dose-dependent increase in human β -cell proliferation with a significant 2-fold increase at the highest OPG dose. Further studies to examine the effects and molecular mechanisms of OPG action are underway. Taken together, our studies reveal OPG to be a novel downstream target of lactogens, which independently enhances human β -cell proliferation and survival, making it one of the few factors with direct beneficial effects on the human β -cell.

Sources of Research Support: DK078060 and DK072264.

Nothing to Disclose: NGK, AO, AM, CC, RCV

Pub # P2-489

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Beta Cell Biology (1:30 PM-3:30 PM)

Title Bmal1 Is Required for Normal β -Cell Mitochondrial Energy Metabolism and Insulin Secretion

Author String J Lee, M-s Kim, R Li, V Liu, Z Fang, DL Nelson, L Fu, DD Moore, K Ma, VK Yechoor
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Body Circadian misalignment, as occurs in shift workers, is associated with diabetes, obesity and metabolic syndrome, while sequence variations in circadian genes have been associated with metabolic syndrome and diabetes in population studies. Though the role of the circadian clock in glucose homeostasis has been reported, its role in β -cell function is not fully understood.
To decipher the role of the circadian clock in β -cell function, we studied global Bmal1 KO mice (Bmal1^{-/-}) and β -cell specific KO mice (β -cell Bmal1^{-/-}). β -cell Bmal1^{-/-} mice were generated by crossing floxed Bmal1 mice (Bmal1^{F/F}) and Rat insulin promoter-cre (Rip-cre) mice. β -cell Bmal1^{-/-} mice display an absence of nuclear Bmal1 protein only in β -cells, but not SCN or other tissues. They have an intact central clock as demonstrated by normal circadian wheel running activity. Using *ex vivo* and *in vivo* studies from these two animal models, we show that these mice develop diabetes secondary to a complete loss of the first phase of glucose-induced insulin secretion (GSIS). A normal insulin tolerance test excluded peripheral insulin resistance in Bmal1^{-/-} and β -cell Bmal1^{-/-}. There was no difference in Glut2, and GCK expression level in isolated islet from those mice. Depolarizing the membrane and activating Ca⁺⁺ entry by L-Arginine, KCl and Glibenclamide treatments revealed insulin exocytosis to be normal. We demonstrate that this defect is primarily because of impaired mitochondrial function due to defective glucose-induced changes in mitochondrial membrane potential gradient and ATP production. We found an up-regulated Ucp2 as the key factor that uncoupled the mitochondria and impaired GSIS, with deletion of Bmal1 in β -cells. We also demonstrate that Bmal1 regulates the level of antioxidant gene, sestrin2, in β -cells such that in Bmal1^{-/-} mice there is decreased sestrin2 expression with resultant accumulation of reactive oxygen species, a strong inducer of Ucp2 expression. Inhibition of Ucp2, in isolated islets, leads to a rescue of the glucose-induced ATP production and insulin secretion. These results demonstrate that Bmal1 regulates mitochondrial energy metabolism to maintain normal β -cell function and glucose stimulated insulin secretion.
An understanding of the underlying mechanisms could lead to potential therapies to improve islet function as part of a strategy to prevent and treat diabetes effectively, especially in the setting of circadian misalignment.

Nothing to Disclose: JL, M-SK, RL, VL, ZF, DLN, LF, DDM, KM, VKY

Pub # P2-490

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Beta Cell Biology (1:30 PM-3:30 PM)

Title Glucagon-Like Peptide-1 Promotes Beta-Cell Proliferation and Improves Glycemic Control in a Murine Model of Neonatal Diabetes

Author String MA Spinner, I Papagiannis, C Dai, AC Powers
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Body Glucagon-like peptide-1 (GLP-1) has been shown to potentiate insulin secretion, promote beta cell proliferation, and reduce apoptosis. To test the hypothesis that GLP-1 would preserve beta cell function and improve glycemic control in a murine model of neonatal diabetes, we examined the effects of a GLP-1 agonist (exendin-4) on Akita mice, which are characterized by severe and progressive hyperglycemia. Upon weaning, wild type and Akita littermates were given daily intraperitoneal injections of exendin-4 or PBS for a four-week period. Random blood glucose (BG) was measured twice per week, and fasting BG, plasma insulin, and plasma glucagon were measured at the end of the treatment period following a six-hour fast. In pancreatic cryosections, beta cell proliferation was assessed by Ki67 labeling and apoptosis by TUNEL staining. Prior to treatment, there was no significant difference in BG between mice assigned to receive control vs. GLP-1 (303 ± 17 vs. 356 ± 31 mg/dl; $n = 7-12$). Three to four days after initiation of treatment, BG further increased in the control group but was reduced in GLP-1 treated mice (329 ± 27 vs. 215 ± 20 mg/dl; $p < 0.01$). After four weeks of treatment, the random BG (524 ± 17 vs. 407 ± 22 mg/dl; $p < 0.01$) and fasting BG (508 ± 18 vs. 372 ± 30 mg/dl; $p < 0.01$) were lower in the GLP-1 treated group. GLP-1 treated mice had higher fasting plasma insulin (corrected for glucose) than the control group ($p < 0.05$), but fasting glucagon levels were similar. Beta cell proliferation, but not apoptosis, was greater in the GLP-1 treated group (1.01 ± 0.02 vs. $1.43 \pm 0.06\%$; $p < 0.001$). The abnormal islet morphology that characterizes the Akita mouse was not improved by GLP-1 treatment. The initial reduction in BG and the maintenance of reduced BG levels suggest both an acute and chronic effect of GLP-1 in improving glycemic control, including acute stimulation of insulin secretion and stimulation of beta cell proliferation. These findings suggest some therapeutic benefit of a GLP-1 agonist in preserving beta cell function and reducing hyperglycemia in neonatal diabetes.

Nothing to Disclose: MAS, IP, CD, ACP

Pub # P2-491

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Beta Cell Biology (1:30 PM-3:30 PM)

Title Plasma Prolactin Levels May Affect the Development of Postnatal Rat Islets

Author String M Argente-Pla, C Aguayo-Mazzucato, A Marinelarena, JF Merino-Torres, A Sharma, GC Weir, S Bonner-Weir
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Body Glucose-induced insulin secretion is poor in neonatal β -cells but gradually increases over the first postnatal month. Since prolactin (PRL) *in vitro* has been shown to induce β -cell proliferation and to increase insulin secretion in neonatal islets, we hypothesized that prolactin may be a physiological modulator of islet functional maturity *in vivo*. Our aim was to characterize plasma PRL levels and islet expression of PRL receptors at different neonatal ages and in response to ligand.

Plasma PRL levels of female Sprague-Dawley rats from birth (P0) to young adults (7 weeks) were measured with ELISA. Expression of two prolactin receptors (PRLR) isoforms, the long with known signaling activity and the short considered as a dominant-negative modulator, was measured by qPCR in isolated islets over the same time course. Islets isolated from P10 and P17 (n=2 pools from 10 rats at each age) were cultured 5 days in RPMI 1640 + 10% charcoal-stripped FBS with or without rhPRL (500 ng/ml) and analyzed for PRLR expression.

We found that neonatal plasma PRL levels are significantly lower than adult, being only about 50% through P15 and only reaching adult values by P21. The pattern of plasma PRL levels were closely followed by expression of the long isoform of the receptor: PRLR^{long} mRNA reached adult levels only by P21 whereas that of PRLR^{short} was at adult level by P7, 2 fold higher by P21 and 8 fold higher at P28. After adjusting for the amplification efficiency of the PRLR primers (-2,66 for PRLR^{long} and -3,15 for PRLR^{short}) the relative level of PRLR^{long} mRNA were found to be higher than PRLR^{short} at all time points examined. Culturing P10 islets with PRL induced a 2.5-fold increase in both PRLR^{long} and PRLR^{short} mRNA compared to control suggesting a positive feed forward mechanism. Yet under similar conditions there was no change in either PRLR mRNA in P17 islets compared with controls.

These data suggest that, given the parallel increase of PRL and the functional PRLR^{long} isoform, most of the physiological effects of this hormone upon the developing islet would be between P15 and P21 after life. Further studies will be needed to determine the precise role of PRL as a physiological factor during postnatal islet maturation.

Nothing to Disclose: MA-P, CA-M, AM, JFM-T, AS, GCW, SB-W

Pub # P2-492

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Beta Cell Biology (1:30 PM-3:30 PM)

Title Implication of Corticotropin-Releasing Hormone on INS-1 and Pancreatic Islets

Author String J Schmid, B Ludwig, C Ziegler, A Steffen, M Brendel, M Ehrhart-Bornstein, SR Bornstein
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Body Hypothalamic corticotropin-releasing-hormone (CRH) is a major regulator of the hypothalamic-pituitary-adrenal axis. This axis is balanced among others by the negative feedback effects of glucocorticoids (GC). GCs are referred to as diabetogenic due to their stimulating effect on glucose production and insulin resistance in pancreatic β -cells. Therefore, an imbalance or overexposure to GC leads to type-2 diabetes. CRH and GC mediate their effects through common intracellular receptors. GC access to intracellular receptors is regulated by 11β -hydroxysteroid-dehydrogenase (11β -HSD). Its two isoforms (11β -HSD-1, 11β -HSD-2) catalyze the interconversion of active GCs and their inactive metabolites.

In the present study we asked if pancreatic islets and insulinoma cells (INS-1) respond directly to CRH by interference with 11β -HSD activity and levels of GC.

First, in vitro studies demonstrated that CRH and its receptor CRH receptor type 1 are expressed at the mRNA and protein levels in INS-1, rat and human islets. Second, we found expression of both 11β -HSD isoforms in INS-1, rat and human islets.

CRH treatment significantly decreased the mRNA level of 11β -HSD-1 and increased the expression of 11β -HSD-2 as measured by quantitative RT-PCR. Specific enzyme activity was analyzed by measuring the production of active GC. Following CRH treatment, active GC levels were significantly reduced indicating an overbalance in favor of 11β -HSD-2 activity. Furthermore, stimulation with CRH resulted in significantly increased total insulin content, thus contributing to the restoration of normoglycemia. Moreover, CRH-receptor activation caused a significant increase in cell proliferation and reduced cell apoptosis.

We suggest that CRH plays an important role in glucose homeostasis and the regulation of β -cell mass. If so, CRH could have implications as a therapeutic tool in patients with metabolic dysfunction and type-2 diabetes.

Nothing to Disclose: JS, BL, CZ, AS, MB, ME-B, SRB

Pub # P2-493

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Beta Cell Biology (1:30 PM-3:30 PM)

Title Short-Term Very-Low-Calorie Diet in Obese Females Improves β -Cell Function

Author String JP Dunn, BW Patterson, P Marks-Shulman, J Kaiser, NN Abumrad
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Body Very low calorie diets (VLCD) (<1000 Kcal/d) are often used during medical weight loss and are standard early after bariatric surgery. In patients with type 2 diabetes mellitus (DM) glycemic control improves rapidly after bariatric surgery. We hypothesized that caloric restriction before clinically significant weight loss contributes to the improvement in glucose metabolism. We studied 7 obese (112 ± 19 kg, BMI 42 ± 7 kg/m²), non-diabetic, females. At baseline and after 10 days of a VLCD, subjects underwent 5 hour 75 gm oral glucose tolerance test (OGTT). The VLCD consisted of ~35% of basal metabolic needs (composition 61% CHO, 33%pro, 6%fat, CHO 96-120 gm/d) in liquid shakes and low calorie vegetables. Insulin sensitivity and beta cell indices were calculated by OGTT minimal model technique. Wilcoxon-signed ranks tests were used for paired analyses. Data are presented as mean \pm SD and as, baseline vs. post-diet, respectively. All subjects lost weight (112 ± 19 kg vs 110 ± 18 kg, $p=0.018$) and BMI (42 ± 7 vs 41 ± 7 kg/m², $p=0.001$) decreased significantly. Neither fasting plasma glucose (101 ± 9 vs 93 ± 13 mg/dl, $p=0.127$) nor insulin (23 ± 14 vs 15 ± 6 [micro]U/ml, $p=0.075$), nor mean insulin sensitivity (Si) which decreased by ~28% (4.3 ± 2.8 vs 3.1 ± 1.5 10⁻⁴ min⁻¹/[micro]U/ml, $p=0.063$) reached significance post-diet. However, overall disposition index (DI), a measure that adjusts insulin sensitivity for insulin secretion, worsened post diet (11.4 ± 5.3 vs 8.5 ± 4.0 10⁷ min⁻²/[micro]U/ml, $p=0.043$), while dynamic β -cell responsivity doubled from baseline post-diet (11.0 ± 11.4 vs 21.9 ± 10.4 10¹¹, $p=0.028$). VLCD for 10 days causes a small amount of weight loss. Our findings do not support that insulin sensitivity improves with VLCD for 10 days. However, dynamic β -cell responsivity, which represents insulin response to maximum glucose, improved dramatically. Early improvements in pancreatic β -cell responsivity may contribute to the improvement in glycemic control that occurs with VLCD before large amounts of weight loss.

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Nothing to Disclose: JPD, BWP, PM-S, JK, NNA

Pub # P2-494

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Beta Cell Biology (1:30 PM-3:30 PM)

Title Local Regulation of Thyroid Hormone Action in Postnatal Rat Islets

Author String C Aguayo-Mazzucato, AM Zavacki, A Marinelarena, J Hollister-Lock, A Marsili, A Sharma, PR Larsen, S Bonner-Weir
Joslin Diabetes Center, Boston, MA; Brigham and Women's Hospital, Boston, MA

Body The functional maturation of postnatal β -cells is a complex physiological process that occurs over the first postnatal month. Previously we showed that increasing thyroid hormone (TH) levels is a physiological way to induce glucose responsiveness of insulin secretion in immature islets. By altering the *in vivo* TH status, we were able to accelerate islet development with T₃ supplementation and delay these processes with methimazole (MMI). However, systemic changes in TH status can be regulated by local changes in T₃ concentrations due to deiodinases in target tissues that activate TH and pour it into the general circulation (*Dio1*), increase intracellular T₃ levels (*Dio2*) or inactivate (*Dio3*) the ligand. Changing levels and isoforms of TH receptors (THRA and THRB) is another way to regulate local TH effects. To understand the mechanisms that influence TH effects in neonatal islets, we examined the time course (P2-P28) of deiodinase and TH receptor isoform expression in islets and how their expression was affected by *in vivo* TH modulation. At P10 *Dio1* mRNA increased to above adult, while surprisingly *Dio2* and *Dio3* both decreased from elevated levels at birth to adult levels by P10; since they have opposite effects, the possibility of their being in different islet cell types should be explored. By immunostaining, THRA was the main isoform expressed at earlier ages while THRB is the main expressed isoform in adults. The variation in pattern of TH receptors level and localization can be used as a reference to evaluate the islet maturation. *In vivo* T₃ treatment from birth until P7 resulted in significantly increased *Dio1* and *Dio3* mRNA in pancreatic islets, which could lead to decreased local T₃ concentrations by inactivating the hormone and diverting it to the general circulation. Treatment with MMI until P21 lowered *Dio1* and *Dio3* and elevated *Dio2* mRNA as expected: *Dio2* is negatively regulated at the transcriptional level as well as at the post-transcriptional level by TH. Changes in TH status were also reflected in the TH receptor patterns. P7 Islets from T₃-treated animals had staining patterns consistent with older ages while pancreas from P15 and P21 MMI-treated animals retained the staining profile of younger animals. In conclusion, we show local regulation of active T₃ concentrations and action, as shown in other systems, could be key to maturation of neonatal islets.

Nothing to Disclose: CA-M, AMZ, AM, JH-L, AM, AS, PRL, SB-W

Pub # P2-495

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Beta Cell Biology (1:30 PM-3:30 PM)

Title Ductal Heterogeneity Suggests a Subpopulation of Ductal Cells as Postnatal Pancreatic Progenitors in Human and Mouse

Author String L Ouziel Yahalom, C Cavelti-Weder, M Zalzman, C Keyzer, W-C Li, L Guo, A Sharma, J Lei, J Markmann, S Bonner-Weir
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Body Both self-duplication of preexisting b cells and the differentiation of new b cells from pancreatic progenitors (neogenesis) can expand b cells in normal postnatal growth and in response to injury. Understanding the molecular mechanisms involved in pancreatic regeneration may lay a basis for b cell replacement therapy for diabetes. Neogenesis from pancreatic duct progenitors cells has been supported by many lines of evidence including two lineage-tracing studies: the first with activation of $ngn3^+$ cells within the pancreatic duct epithelium after partial duct ligation in adult mice; the second, using a duct-specific promoter in a Cre-lox system showing ductal origin of all pancreatic cell types in neonates and after partial duct ligation. However, recent lineage tracing studies using promoters of the transcription factors of embryonic pancreatic progenitors Hnf1b and Sox9 suggested that pancreatic duct cells give rise to b cells only during embryogenesis but not after birth or after injury. Our interpretation is that within the adult ductal epithelium there were cells expressing low ($Hnf1b^{low}$) or undetectable ($Hnf1b^{undetectable}$) levels of Hnf1b that were not marked by Cre excision and could act as pancreatic progenitors.

We found heterogeneous expression of both Hnf1b and Sox9 proteins throughout the ductal tree in human and mouse pancreas. The intensity and proportion of the expressing populations varied within the categories of ducts and not all cells express both. Using nuclear FACS analysis of nuclear-immunostained purified duct cells in mouse (n=3 pooled samples), $77.3 \pm 2.6\%$ duct cells were Hnf1b+ and $77.4 \pm 2.4\%$ Sox9+; about half of the positive cells expressed high intensity. In tissue from 8 human donors, $90.2 \pm 2.0\%$ of the ductal cells expressed Hnf1b and $64.9 \pm 7.4\%$ Sox9, with $23.3 \pm 2.0\%$ Hnf1b^{high} and $15.4 \pm 4.1\%$ Sox9^{high}. The lower proportion of high intensity of Hnf1b and Sox9 expression in humans compare to mouse may reflect species difference in reliance on neogenesis in the adult for b cell expansion. In regenerating rodent models, the expression level of Hnf1b seems to reflect the state of differentiation of the duct cells: during regeneration a greater portion are $Hnf1b^{low/undetectable}$ and $Ngn3^+$ cells were $Hnf1b^{undetectable}$. The heterogeneity of Hnf1b and Sox9 expression, markers of pancreatic progenitors, within the pancreatic ductal tree suggests subpopulations of cells with different potential to act as pancreatic progenitors.

Nothing to Disclose: LOY, CC-W, MZ, CK, W-CL, LG, AS, JL, JM, SB-W

Pub # P2-496

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Beta Cell Biology (1:30 PM-3:30 PM)

Title Reg2 Prevents ER-Stress-Induced Scythe- and AIF-Mediated Apoptosis in Mouse Insulinoma Cells

Author String L Liu, J-L Liu, L Larose, CB Srikanth
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Body We have previously shown that Reg2, a member of the regenerating protein family, attenuates streptozotocin- and cytokine-induced apoptosis by inhibiting the intrinsic caspase-mediated cytotoxic signalling in MIN6 mouse insulinoma cells. In this study we investigated if Reg2 protects against ER stress-induced caspase-independent apoptotic signaling. It has been reported that upon induction of ER stress, Scythe (BAT-3/HLA-I associated transcript 3/Bag6), a regulator of apoptosis, translocates to the mitochondria where it increases AIF (apoptosis inducing factor) stability and promotes the release of its cleaved product into the cytosol. Translocation of AIF from the cytosol to the nucleus leads to endonuclease activation and DNA damage whereas presence of Scythe in the nucleus protects the DNA from degradation. In order to explore the possibility that Reg2 may regulate ER stress-induced changes in Scythe and AIF we compared the effect of thapsigargin (Tg) treatment in MIN6 cells stably transfected with pcDNA3.1 alone (MIN6-VC) or encoding HA-tagged Reg2 (MIN6-Reg2). Cells were incubated in the absence and presence of 1 mM Tg. ER stress was monitored as a function of time of Tg treatment by evaluating the phosphorylation of eIF2a at Ser⁵¹, and proapoptotic events were assessed by following changes in AIF and Scythe in the cytosol and the nucleus. Tg induced a robust, time-dependent increase in p-eIF2aSer⁵¹ in MIN6-VC, but not in MIN6-Reg2 cells. Scythe was present in the cytosol to a greater extent in Tg-treated MIN6-VC cells compared to MIN6-Reg2 cells while its nuclear presence was higher in MIN6-Reg2 cells. The cytosolic presence of AIF was higher in cells expressing Reg2 compared to VC cells. By contrast, AIF was present in the nucleus of VC, but not in Reg2 expressing cells. Taken together, these observations show that Reg2 protects against ER stress-induced apoptosis in mouse insulinoma cells at multiple levels that include attenuation of ER stress response and AIF-mediated apoptosis, stimulation of nuclear import of Scythe and inhibition of AIF translocation to the nucleus from the cytosol. Thus, the present findings demonstrate for the first time that Reg2 protects mouse insulinoma cells from Scythe-mediated caspase-independent pro-apoptotic events induced by ER stress.

Nothing to Disclose: LL, J-LL, LL, CBS

Pub # P2-497

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Beta Cell Biology (1:30 PM-3:30 PM)

Title Pancreatic Islet β -Cell-Specific Overexpression of Reg3 β Protein Causes Decreased GLUT2 Expression and Deteriorated Diabetes Induced by High-Fat Diet

Author String X Xiong, B Li, L Larose, J-L Liu
McGill University Health Centre, Montreal, Canada

Body Reg family proteins have been implicated in pancreatic islet cell proliferation, survival and regeneration. The expression of Reg3 β (pancreatitis-associated protein) is highly induced in experimental diabetes and acute pancreatitis but its precise role has not been established. Through knockout studies, this protein was shown to be mitogenic, anti-apoptotic and anti-inflammatory in the liver and pancreatic acinars (1). In order to test whether it can promote islet cell growth or survival against experimental damage, we developed β -cell-specific overexpression using rat insulin I promoter. In previous report, Reg3 β protein induced the expression of several pro-islet genes, protected mice against streptozotocin-induced diabetes, but did not affect normal β -cell mass and insulin secretion (2). To further verify its effect, we established high-fat diet (HFD)-induced obesity and diabetes. Both RIP-I/Reg3 β and wild-type mice gained weight (~50% increase vs. chow diet in 10 wk) rapidly upon feeding the diet. Blood glucose level started to elevate in wild-type mice after 4 wk, reaching a maximum 238 mg/dL by 10 wk; RIP-I/Reg3 β mice exhibited a similar pattern of hyperglycemia except the level was elevated faster and higher, peaked at 330 mg/dL, indicating worsened T2D. We also recorded impaired glucose tolerance in wild-type mice on HFD and a further deterioration in RIP-I/Reg3 β mice; and more severe insulin resistance in the mice. Pancreatic immunohistochemistry revealed obvious and similar level of compensation of the islet size in all animals after HFD; however, the intensity of insulin staining was lower implying an exhausted secretion and/or depleted storage in RIP-I/Reg3 β mice. In order to characterize the underlying mechanism, we demonstrated that Reg3 β overexpression caused decreased normal GLUT2 expression in the β -cells at the levels of mRNA, protein and immunohistochemistry; after HFD, GLUT2 distribution in the transgenic β -cells was disrupted and the staining was much diminished on cell membrane. On the other hand, the detrimental effect was unlikely caused by increased foreign protein and ER stress in the β -cells, as eIF2 α phosphorylation was not elevated. Our result suggests that Reg3 β is unlikely a growth factor that promotes β -cell compensation against T2D; nor a protector against β -cell death upon HFD feeding. It seems to cause a clear inhibition on GLUT2 expression, which may explain the early onset and deteriorated diabetes in RIP-I/Reg3 β mice.

1. HT Lieu, et al. Hepatology 2006; 44: 1152
2. X Xiong, et al. Am J Physiol - Endo Metab 2011 (in press)

Sources of Research Support: Canadian Institutes of Health Research; National Science and Engineering Research Council of Canada; Fonds de la recherche en santé Quebec.

Nothing to Disclose: XX, BL, LL, J-LL

Pub # P2-498

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Beta Cell Biology (1:30 PM-3:30 PM)

Title Ductal Origin of Regenerated Pancreas after Partial Pancreatectomy in Mice Using Lineage Tracing

Author String L Guo, W-C Li, A Sharma, S Bonner-Weir
Joslin Diabetes Center, Boston, MA

Body The regeneration model of partial pancreatectomy (Px) in adult rodents has enhanced b cell proliferation and formation of whole new lobes of pancreas with newly formed islets (neogenesis). We have previously shown in adult rats with Px that these new lobes expand from the common pancreatic duct, with proliferation of ductal cells and branching morphogenesis. Ductal cells in these well-defined proliferating areas, termed regenerating foci, progress through the embryonic cascade of transcription factors to differentiate into acini and islets having normal pancreatic morphology. We have now replicated these findings in the mouse Px model. Further to determine the origin of the acini and islets in the newly formed lobes we performed Px in bigenic mice with a lineage tracing reporter (ROSA26EYFP) and inducible duct-specific carbonic anhydrase II (CAII)-CreER2 transgene.

At 4 weeks age, tamoxifen pellets were implanted and then at 8 week, Px or sham surgery was performed. In tamoxifen implanted unoperated/sham bigenic mice, many but not all ductal epithelial cells of the common pancreatic duct (CPD), and many cells in smaller ducts, were EYFP⁺; however, acini and islets were negative for EYFP. At Px+5 days, regenerating foci were detected in the Px bigenic mice. In young foci, some of the branching ductules were marked with EYFP. In mature foci, small ductules, acinar cells and islets were labeled. In the remnant area EYFP positive cells were only detected in the ducts. Pancreatic tissue harvested a Px+3 weeks confirmed the contribution of ductal cells to the regeneration of new pancreatic lobes. In these pancreases, EYFP positive islets and acini were detected, indicating that these cells originated from the EYFP labeled ductal cells. In control mice (CAII-CreER2-ROSA26EYFP mice without tamoxifen treatment) no EYFP positive cell was detected, even after Px. In conclusion, the mouse model of 70-80% Px triggers extensive neogenesis as well as enhanced replication for regeneration. The lineage tracing experiment show that ductal cells in adult mice can serve as pancreatic progenitors during this regeneration.

Nothing to Disclose: LG, W-CL, AS, SB-W

Pub # P2-499
Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Beta Cell Biology (1:30 PM-3:30 PM)
Title Unacylated Ghrelin Fragments Promote Survival of Pancreatic β -Cells and Human Pancreatic Islets and Influence Glucose and Lipid Metabolism in C2C12 Myotubes and 3T3-L1 Adipocytes
Author String R Granata, F Settanni, D Gallo, C Grande, R Nano, A Baragli, M Taliano, MF Brizzi, L Piemonti, T Aribat, AJ van der Lely, E Ghigo
University of Turin, Turin, Italy; Alizé Pharma, Ecully, France; Erasmus Medical Center, Rotterdam, Netherlands; San Raffaele Scientific Institute, Milan, Italy

Body The 28-aminoacid peptide unacylated ghrelin (UAG), although unable to bind the acylated ghrelin (AG) receptor GHS-R1a, exerts a wide range of biological effects. Indeed UAG, similarly to AG, protects in vitro β -cells and human pancreatic islets against apoptosis, stimulate their proliferation and enhances glucose-induced insulin secretion at physiological concentrations (1). In vivo, UAG prevents diabetes at adult age in streptozotocin (STZ)-treated neonatal rats by reducing blood glucose, and increasing β -cell mass and insulin secretion (2). Here, we investigated the potential survival effects of UAG fragments in β -cells and human pancreatic islets and their metabolic actions in C2C12 muscle cells and 3T3-L1 adipocytes. UAG fragments either included or excluded serine-3, the site for ghrelin octanoylation essential for binding to GHS-R1a. UAG fragment comprising aminoacids 6 to13 (6-13) displayed survival and antiapoptotic actions equal to or even greater than full length UAG (1-28) in both β -cells and human islets, upon either treatment with cytokine synergism or glucolipotoxicity. In addition, although to a lesser extent, fragments 1-14 and 1-18 retained all the protective effects of UAG 1-28, including stimulation of glucose-induced insulin secretion. Conversely, fragments 8-13 and 8-12 showed reduced activity. UAG 6-13, which showed the best protective effects in vitro, also prevented diabetes at adult age in STZ-treated neonatal rats, by decreasing blood glucose and increasing blood and pancreatic insulin levels, similarly to UAG. In addition, UAG 6-13 reduced isoproterenol-induced lipolysis in 3T3-L1 cells and human subcutaneous adipocytes. It also increased glucose and free fatty acid uptake in both 3T3-L1 adipocytes and C2C12 myotubes, in either absence or presence of insulin. These results indicate that UAG fragments, particularly 6-13, besides exerting protective effects in pancreatic β -cells and human islets, also influence glucose and lipid metabolism in adipocytes and myotubes. Therefore, UAG 6-13 appears to exhibit the same pharmacological profile and therapeutic potential in metabolic dysfunctions and diabetes, as its parent molecule.

(1) Granata R et al., J Mol Endocrinol. 2010;45:9

(2) Granata R et al., Endocrinology. 2007;148:512

Sources of Research Support: Univesity of Turin (Ex-60% 2007); Regione Piemonte (2007, 2009); Compagnia di San Paolo; Alizé Pharma and by Studio delle Malattie Endocrino Metaboliche (SMEM) Foundation, Turin, Italy.

Nothing to Disclose: RG, FS, DG, CG, RN, AB, MT, MFB, LP, TA, AJvdL, EG

Pub # P2-500

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Beta Cell Biology (1:30 PM-3:30 PM)

Title Identification of a Novel GPCR Mediating Anti-Diabetic Effects of Alpha-1-Antitrypsin

Author String R Kumar, A Balhuizen, A Soni, A Salehi
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Body The inflammatory mediators seem to play an important role in the β -cell failure/apoptosis in type 1 and type 2 diabetes. Therefore therapeutic approaches targeting the inflammatory processes in the islets could be a significant new development in type 1 and 2 diabetes. We hypothesized a potential interaction between Alpha-1 anti trypsin (AAT) and new novel GPCR signaling might relate pathophysiologically to inflammation in the setting of Diabetes. Therefore objective of this study was to identify the new anti-diabetic drug target GPCR expression on pancreatic islets and investigate the potential interaction between proinflammatory effects of a novel GPCR and anti inflammatory effect of Alpha-1-antitrypsin on Human pancreatic islets. In this study we observed proinflammatory role of GPCR in pancreatic islets and human cellline HeLa cells. We showed that activation of GPCR by cytokines or GPCR ligand induces beta cell apoptosis. In addition, we demonstrated that AAT significantly reduced GPCR expression and increase pancreatic islet survival and proliferation. Thus; these data suggest that this new novel GPCR could represent a possible new therapeutic target in diabetes. GPCR expression was analyzed by confocal microscopy, western blot and qPCR in human pancreatic islets. Hormone secretion and cAMP content in islets were determined with RIA and apoptosis with Annexin-V method. Binding study of AAT to GPCR was done on GPCR transfected HeLa cells; AAT was labeled with I125 measured with gamma counter. Our Confocal microscopy revealed that this novel GPCR expressed in alpha, beta and delta cells in human islets. GPCR mRNA and protein expression was markedly higher in diabetic vs non diabetic donors ($p < 0.01$). Dose-response studies of AAT in isolated islets at 12 mM glucose showed insulintropic effects on pancreatic islets. Cytokine-induced (IL-1 β 100ng/ml, TNF α and INF 125ng/ml) apoptosis in islets, cultured for 24 h at 5 mmol/l glucose was significantly reduced by AAT treatment ($p < 0.001$). The result of the binding study shows that AAT significantly binds to new novel GPCR and mediates its insulintropic and anti apoptotic effects. In view of these novel finding we suggest that drugs that can selectively inhibit the activity this novel GPCR, represent a promising frontier in diabetes mellitus therapy.

Nothing to Disclose: RK, AB, AS, AS

Pub # P2-501

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Beta Cell Biology (1:30 PM-3:30 PM)

Title Dexamethasone as a Regulator of β -Cell Maturation

Author String AM Marinelarena, C Aguayo-Mazzucato, S Bonner-Weir
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Body Neonatal rodent β -cells lack the characteristic glucose-responsive insulin secretion (GSIS) of mature functional β -cells but gain this function over the first postnatal month. We have studied possible physiological factors regulating this process and previously showed thyroid hormone to be such a factor and that its induction of GSIS was mediated through MafA. Another factor may be corticosterone: this main glucocorticoid in rodents increases during the second week of postnatal life, a time when islet expression of key transcription factors Pdx1, NeuroD1 and MafB peak. The circulating plasma corticosterone level in rodents increases moderately to 0.2 ng/ml of plasma corticosterone by day 15, surges to 0.8 ng/ml by day 21 and increases to adult levels thereafter. However, the glucocorticoid receptor expression in rodent islets remains unchanged during the neonatal period suggesting any gene expression changes observed throughout the postnatal period may result from increased corticosterone levels rather than its receptor expression. We tested whether glucocorticoids played a role in β -cell maturation using an *in vitro* model to examine the direct effects of dexamethasone (DEX), a glucocorticoid analog, on the gene expression of immature rodent islets. P9 rodent islets were cultured for 48 hours in RPMI 1640 with 10% charcoal stripped-fetal bovine serum (CS-FBS) with either DEX (10^{-11} M), DEX (10^{-11} M) + RU486 (10^{-5} M), an antagonist to the glucocorticoid receptor, or untreated (controls). After culture with DEX, MafA, NeuroD1 and Preproinsulin mRNA were significantly increased (1.5-fold), and Pdx1, Glut2 and Glucokinase mRNA tended to increase. The specificity of glucocorticoid action was shown by the lack of mRNA changes in islets cultured in DEX (10^{-11} M) + RU486 (10^{-5} M) compared to controls. These results suggest that glucocorticoids differentially regulate key transcription factors and critical phenotypic genes of the β -cell. *In vivo* these effects may however be modulated by counter effects of other circulating factors. In conclusion these studies define another component that contributes to the maturation process of functional β -cells. A better understanding of the physiological changes driving the molecular mechanisms of the development of glucose-responsive β -cells could be applied to *in vivo* generation of functional β -cells for replacement therapy for T1DM.

Nothing to Disclose: AMM, CA-M, SB-W

Pub # P2-502

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Beta Cell Biology (1:30 PM-3:30 PM)

Title Exendin-4 Protects MIN6 Cells from Endoplasmic Reticulum Stress-Induced Apoptosis

Author String W Chen, L Wang, Y Wang, Z Chen, X Liu, X Liu, L Liu
Fujian Institute of Endocrinology Union Hospital of Fujian Medical University, Fuzhou, China

Body The aim of this study was to explore the effect and mechanism of exendin-4 on t-BHP-induced apoptosis in pancreatic β -cells. Murine MIN6 pancreatic β -cells were treated with exendin-4 in the presence or absence of tert-butyl hydroperoxide (t-BHP). Cell survival was assessed by MTT staining. The percentage of apoptotic cells was determined by fluorescence microscopy analysis after Hoechst/PI staining and a flow cytometric assay after annexin-V-FITC/PI staining. The activity of caspase-3 was determined using a caspase-3 activity kit. Expression of P-IRE1 α , IRE1 α , C-Jun N-terminal kinase (JNK), P-JNK, C-JUN, and P-C-JUN was detected by western blotting. Exendin-4 was found to inhibit t-BHP-induced apoptosis in pancreatic β -cells by down-regulating caspase-3 activity. Exendin-4 also inhibited the endoplasmic reticulum transmembrane protein IRE1 α , the apoptosis-related signaling molecule JNK, and c-Jun activation. Our findings suggest that exendin-4 ultimately reduces t-BHP-induced β -cell apoptosis. ER-stress-JNK-c-Jun signaling is involved in the exendin-4-mediated modulation of β -cell apoptosis.

Nothing to Disclose: WC, LW, YW, ZC, XL, XL, LL

Pub # P2-503

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Beta Cell Biology (1:30 PM-3:30 PM)

Title Potential Role of Ngn3 in Regulation of β -Cell Proliferation during Pregnancy

Author String C Huang
University of Calgary, Calgary, Canada

Body There is endocrine pancreas plasticity such that β -cell mass can increase significantly in adaptation to increased insulin demand (i.e. during pregnancy and obesity). Whether this increase is a result of β -cell proliferation only or whether there is contribution from β -cell regeneration (i.e. differentiation from progenitors) is unclear. The existence of β -cell progenitors in adult pancreas is controversial. While many have generated insulin-secreting cells from either putative progenitor cells or trans-differentiation from other cell types, with ductal cells as the most commonly cited source of new β -cells, recent lineage tracing studies suggested that most β -cells in adult pancreas are derived from proliferation of pre-existing β -cells. We found that during pregnancy, there is an increase in the number of small islets, i.e. clusters of <5 β -cells in comparison to the non-pregnant pancreas, suggesting β -cell neogenesis. Ngn3 is a transcription required for development of endocrine cells, and since it is mainly expressed in fetal pancreas, it is often used as a marker of β -cell progenitors. The objective of this study is to determine whether β -cell mass expansion of pregnancy involves recruitment of Ngn3-positive endocrine progenitors. Using a transgenic mouse with the entire coding region of Ngn3 replaced by EGFP (enhanced green fluorescent protein), i.e. the Ngn3^{EGFP/+} mouse, we found that Ngn3-positive cells are present in both non-pregnant and pregnant islets. Only few cells in each islet are Ngn3-positive, and the number did not increase during pregnancy. Some, but not all of the Ngn3-positive cell also stain positive for insulin. Using real-time RT PCR, we found that Ngn3 mRNA expression in the islets is reduced in early pregnancy (day 9), which recovered slightly by day 15 of pregnancy, although never quite reach the non-pregnant levels. Most interestingly, this drop in Ngn3 mRNA expression on day 9 of pregnancy was not observed in islets isolated from mice with heterozygous deletion of prolactin receptor (Prlr^{+/-}). Since β -cell number increased most significantly between days 9 and 15 of pregnancy, and the Prlr^{+/-} mice had reduced β -cell number in comparison to the wild type mice, this result suggest that during pregnancy, Ngn3-positive progenitors are recruited to form mature β -cells in a prolactin-dependent manner. Further studies will attempt to isolate these Ngn3-positive cells and differentiate them into insulin secreting cells in vitro.

Sources of Research Support: Canadian Diabetes Association, Alberta Children's Hospital Foundation.

Nothing to Disclose: CH

Pub # P2-504

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Beta Cell Biology (1:30 PM-3:30 PM)

Title Beta-Cell Changes in an Insulin Resistance Model Persist Despite Improved Glycemic Control

Author String I Papagiannis, D Chunhua, G Poffenberger, AC Powers
Vanderbilt University Medical Center, Nashville, TN; VA Tennessee Valley Healthcare System, Nashville, TN

Body Hyperinsulinemia secondary to increased insulin secretion and beta cell hyperplasia are hallmarks of the beta cell adaptation to insulin resistance in mouse models. To assess the contribution of hyperglycemia in the beta cell responses to insulin resistance, we measured beta cell proliferation, islet vascularization, and gene expression in ob/ob mice treated with phlorizin, a SGLT1 inhibitor that blocks the reabsorption of ultrafiltered glucose in the proximal kidney tubule, induces glucosuria, and lowers blood glucose levels in an insulin-independent fashion. Six-week old ob/ob mice, treated with phlorizin (1mg/ml infused continuously via a subcutaneous pump) had higher glucosuria (947±53 vs 307±122 mg/dl, p<0.01, n=14-17) with lower random blood glucose levels (181±9 vs 286±19 mg/dl, p<0.001, n=42-50). Phlorizin-treated mice had significant glucosuria even when their blood glucose levels were normal. Fasting insulin levels at 2 and 4 weeks of treatment were similar in both groups (8.9±2 vs 10.9±2.4 ng/ml and 19.7±3.7 vs 11.9±2.1 respectively, p>0.05, n=6). Beta cell proliferation, analyzed by Ki67 labeling, was similar in treated and control mice (1.2±0.3 vs 1.4±0.1%, p>0.05, n=3). Changes in islet vasculature, analyzed with intravital lectin staining, showed a similar increase in vessel size and decrease in vessel density in both groups (30.4±2.1 vs 26.8±0.9 μm^2 and 2833±112 vs 2793±104 mm^{-2} , p>0.05, n=3). The mRNA levels for insulin, GLUT2, Kir6.2, GK and ER stress genes (such as BiP, p58, GRP94, PDIA4 and CHOP) were similar in both groups. However, the mRNA levels of Pdx-1 and Nkx6.1, which are involved in beta cell development and maturation, were greater in the phlorizin-treated group (1.4±0.09 vs 1±0.06 and 1.42±0.09 vs 1.01 vs 0.09 respectively, p<0.05, n=3). These data indicate that amelioration of hyperglycemia via an insulin-independent pathway has little effect on the adaptive changes of the beta cell to insulin resistance, suggesting these result from a factor related to the insulin resistance rather than hyperglycemia.

Nothing to Disclose: IP, DC, GP, ACP

Pub # P2-505

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diabetes & Glucose Homeostasis: Genetic & Translational Approaches (1:30 PM-3:30 PM)

Title A Haplotype in the Untranslated Gene H19 and Ischemic Heart Disease Risk in Type 2 Diabetes Mellitus

Author String RH Stephens, JP New, WER Ollier, JM Gibson
University of Manchester, Manchester, UK; Manchester Academic Health Science Centre, Salford Royal Foundation Trust, Salford, UK

Body The region of chromosome 11p15.5 comprising the genes for tyrosine hydroxylase (TH), insulin (INS) and IGF2 has been associated with increased obesity and diabetes risk. The untranslated H19 gene lies close to IGF2 and is thought to regulate the expression and activity of the TH-INS-IGF2 gene complex. 82 SNPs and microsatellite repeat across the TH-INS-IGF2-H19 region, 42 of them within the H19 locus or the IGF2-H19 intergenic, were genotyped using Sequenom iPLEX in 751 genomic DNA samples taken from subjects with type 2 diabetes from the Salford Diabetes Collection as previously described (Stephens et al. 2005). Armitage Cochran Trend tests, corrected post-hoc by permutation tests, revealed 3 SNPs to be associated with increased risk of ischemic heart disease (IHD) using the recessive model at the 0.05 level, two in the H19-IGF2 intergenic and one in IGF2. 18 SNPs were shown to form 4 haplotype blocks across the region, the largest block comprising 12 SNPs within 5.5 kb of the H19 locus, using a more stringent modification of the haplotype block detection method of Gabriel et al. (2002). The H19 SNPs formed four haplotypes (freq>0.01) the third most common haplotype (freq: 0.14) was associated with reduced risk of IHD (OR: 0.50, 95% CI: 0.36-0.83) which was significant (χ^2 tests, permutation test-corrected $p = 0.005$, Bonferroni corrected $p = 0.040$, FDR = 0.040). It is concluded that common H19 haplotypes are associated with reduced IHD risk in people with type 2 diabetes.

Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, DeFelice M, Lochner A, Faggart M, Liu-Cordero SN, Rotimi C, Adeyemo A, Cooper R, Ward R, Lander ES, Daly MJ, Altshuler D. *Science* 2002;296:2225-2229

Stephens RH, McElduff P, Heald AH, New JP, Worthington J, Ollier WE, Gibson JM: *Diabetes* 2005; 54:3547-3553.

Sources of Research Support: Salford Royal Hospitals Foundation Trust Research and Development Directorate and Manchester Academic Health Sciences Centre.

Nothing to Disclose: RHS, JPN, WERO, JMG

Pub # P2-506

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diabetes & Glucose Homeostasis: Genetic & Translational Approaches (1:30 PM-3:30 PM)

Title InsVNTR Genotypes in SEARCH Participants: Characterization and Associated Clinical Characteristics

Author String LK Gilliam, C Davis, D Dabelea, JM Lawrence, SM Marcovina, EJ Mayer-Davis, EA Rutledge, DA Standiford, C Pihoker
University of Washington, Seattle, WA; Wake Forest University Health Sciences, Winston-Salem, NC; University of Colorado Denver, Aurora, CO; Kaiser Permanente Southern California, Pasadena, CA; University of North Carolina Gillings School of Global Public Health, Chapel Hill, NC; Salish Kootenai College, Pablo, MT; Children's Hospital Medical Center, Cincinnati, OH; University of Washington, Seattle, WA

Body A variable number of tandem repeats (VNTR) polymorphism in the promoter region of the insulin gene (IDDM2) is associated with type 1 diabetes (T1D). The class I alleles are predisposing and the class III alleles are protective.
We performed insVNTR genotyping in 3043 participants in the SEARCH for Diabetes in Youth, a population-based study of childhood diabetes in the US. Our aims were to describe the association between insVNTR genotypes and diabetes type in an ethnically diverse cohort and to examine the association between insVNTR genotype and BMI in youth with diabetes.
The distribution of insVNTR predisposing (I/I) and protective (III/III) genotypes differed ($p < 0.001$) by clinical diabetes type: 67.3% and 5.4%, respectively, in youth with T1D ($N = 2722$) vs. 36.6% and 24.8% in those with T2D ($N = 295$). The frequencies of I/I, I/III and III/III among participants with T1D by race/ethnicity were as follows: White (71%, 26%, 3%, respectively), Black (20%, 47%, 33%), Hispanic (70% 27%, 3%), and Asian/Pacific islander (81%, 19%, 0%). In T1D, the III/III genotype (vs. I/I) was associated with older age at diagnosis ($9.3 \text{ yrs} \pm 4.2$, vs. 8.0 ± 4.2 , $p = 0.001$) but similar duration-adjusted fasting C-peptide (FCP) levels (1.0 ± 0.9 , vs. 0.9 ± 0.7 , ns). Conversely, in T2D, the III/III genotype (vs. I/I) was associated with lower FCP levels (2.7 ± 1.4 , vs. 4.1 ± 2.6 , $p = 0.005$), but similar age at diagnosis ($13.9 \text{ yrs} \pm 2.5$, vs. 13.5 ± 2.4 , ns). The frequency of III/III genotype was not significantly different in those clinically diagnosed with T1D, regardless of the absence (4.7%) or presence (5.6%) of diabetes autoantibodies (DAA). However, the III/III genotype was less common among those clinically diagnosed with T2D with at least one positive DAA ($n = 34$) (8.8%, vs. 27.0% for DAA-negative T2D participants, $p = 0.022$). In T1D, the III/III genotype (vs. I/I) was associated with lower birth weight ($3299 \text{ gms} \pm 674$, vs. 3447 ± 586 , $p = 0.016$), but similar BMI Z-score (0.7 ± 1.0 , vs. 0.6 ± 0.9 , ns). In T2D, there were no significant differences between III/III vs. I/I in birth weight ($3191 \text{ gms} \pm 790$, vs. 3224 ± 766 , ns) or BMI Z-score (2.1 ± 0.8 , vs. 1.9 ± 0.8 , ns). In summary, the insVNTR genotypes show marked differences by diabetes type in youth with diabetes. The insVNTR III/III genotype was associated with older age at diagnosis and lower birth weight in T1D, and lower FCP levels and DAA-negativity in T2D. There was no clear association of insVNTR genotype with BMI.

Nothing to Disclose: LKG, CD, DD, JML, SMM, EJM-D, EAR, DAS, CP

Pub # P2-507

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diabetes & Glucose Homeostasis: Genetic & Translational Approaches (1:30 PM-3:30 PM)

Title InsVNTR Genotypes in SEARCH Participants: Study of Conferred Risk on Beta-Cell Function over Time

Author String LK Gilliam, C Davis, D Dabelea, JM Lawrence, SM Marcovina, EJ Mayer-Davis, EA Rutledge, DA Standiford, C Pihoker
University of Washington, Seattle, WA; Wake Forest University Health Sciences, Winston-Salem, NC; University of Colorado Denver, Aurora, CO; Kaiser Permanente Southern California, Pasadena, CA; University of North Carolina Gillings School of Global Public Health, Chapel Hill, NC; Salish Kootenai College, Pablo, MT; Children's Hospital Medical Center, Cincinnati, OH; University of Pediatrics, Seattle, WA

Body A variable number of tandem repeats (VNTR) polymorphism in the promoter region of the insulin gene (IDDM2) is associated with type 1 diabetes (T1D). The class I alleles are predisposing and the class III alleles are protective.
Our aim was to describe the association between insVNTR genotypes and beta-cell function over time in study participants with T1D. We performed insVNTR genotyping on samples from 3043 participants from SEARCH for Diabetes in Youth, a population-based study of childhood diabetes in the US. In this report, we analyzed longitudinal data for 524 T1D participants with <18 months duration of disease at baseline and at least one follow-up visit where fasting C-peptide (FCP) or hemoglobin A1c (A1c) were measured, using mixed models.
FCP levels (ng/ml; geometric mean) at baseline (7.6 +/- 4.6 months after diagnosis), visits 1 (21.8 +/- 5.5 months after diagnosis), 2 (34.3 +/- 5.6 months after diagnosis), and 3 (68.4 +/- 5.4 months after diagnosis), were as follows: Class I/I - 0.55, 0.30, 0.20, 0.07; Class I/III - 0.55, 0.33, 0.20, 0.06; Class III/III - 0.69, 0.63, 0.33, 0.06. The FCP was significantly higher in participants with III/III vs. I/I and I/III at visit 1 (p<0.001 for both comparisons) and visit 2 (p=0.004 and p=0.005, respectively), but not at baseline or visit 3. A1c levels (%) at the various timepoints were similar for all three groups: Class I/I - 7.6, 8.2, 8.6, 9.1; Class I/III - 7.5, 8.0, 8.5, 9.3; Class III/III - 7.1, 8.2, 9.2, 9.0 (all comparisons ns).
In summary, in youth with T1D, the protective insVNTR III/III genotype was associated with higher FCP levels at visits 1 and 2 (21.8 to 34.3 months after diagnosis). However, by visit 3 (68.4 months after diagnosis), residual beta-cell function was lost, regardless of insVNTR genotype. InsVNTR genotype did not affect glycemic control, which worsened over time in all participants at a similar rate.

Nothing to Disclose: LKG, CD, DD, JML, SMM, EJM-D, EAR, DAS, CP

Pub # P2-508

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diabetes & Glucose Homeostasis: Genetic & Translational Approaches (1:30 PM-3:30 PM)

Title Diabetes-Associated Variants in *TCF7L2* and *KCNQ1* Modulate the Relationship of Insulin Secretion with Weight and Hyperglycemia in Non-Diabetic Subjects

Author String M Sathananthan, G Smushkin, A Sathananthan, C Dalla Man, C Cobelli, AR Zinsmeister, A Vella
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Body **Background:** Diabetes-associated alleles in *TCF7L2* and *KCNQ1* have been shown to impair the response to an oral glucose challenge. However, there are multiple covariates affecting insulin secretion and action across the spectrum of pre-diabetes. To date it has been difficult to quantify the influence of genetic variation when assessing the impact of covariates such as adiposity and age on insulin secretion and action in non-diabetic subjects.

Objective: To assess the influence of *TCF7L2* and *KCNQ1* genotype on the association of specific covariates with the response to glucose in non-diabetic subjects.

Methods: A population-based sample of 190 individuals with a fasting glucose concentration < 7.0mmol/L were studied after an overnight fast using a 2-hour 7-sample oral glucose tolerance test. Insulin action (S_i) was estimated from plasma glucose and insulin concentrations using the oral glucose minimal model. β -cell responsivity indices were estimated from using the oral C-peptide minimal model. The Disposition Indices (DI) for each individual were calculated by multiplying insulin secretion ($[\phi]$) by S_i . Genotyping ascertained the presence or absence of diabetes-associated alleles at both loci. Generalized linear regression models examining the relationships of DI with pre- and post-challenge glucose, and weight, assessed the influence of genotype on these relationships by incorporating separate genotype by glucose and genotype by weight interaction terms in the models. The models also adjusted for gender.

Results: DI was inversely related to fasting and post-challenge glucose concentrations and with weight. Genotype influenced these relationships ($p=0.02$ for *TCF7L2* by glucose interaction term, and $p=0.04$ for *KCNQ1* by weight interaction term), in both cases an attenuation of the inverse relationships were observed for the CC *TCF7L2* genotype and the CC *KCNQ1* genotype.

Conclusions: Diabetes-associated alleles in 2 loci contribute to the variability in DI in prediabetes. This effect is more apparent in subjects with weights closer to ideal body weight and normal glucose tolerance.

Nothing to Disclose: MS, GS, AS, CDM, CC, ARZ, AV

Pub # P2-509

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diabetes & Glucose Homeostasis: Genetic & Translational Approaches (1:30 PM-3:30 PM)

Title Proteomic and Ingenuity Pathway Analysis of Skeletal Muscle Mitochondria in Type 2 Diabetes Patients Suggests Altered Regulation of Specific Fatty Acid Oxidation and TCA Cycle Enzymes

Author String S Kamath, A Monroy, AO Chavez, MA Wilson, CA Carroll, F Casiraghi, D Tripathy, SE Weintraub, RA DeFronzo, F Folli
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Body Disturbances in fatty acid metabolism are closely associated with obesity and type 2 diabetes (T2DM) leading to lipid accumulation in insulin resistant skeletal muscle. We analyzed highly enriched mitochondrial fraction from 8 lean normal glucose tolerant (NGT; 4M/4F, age 36±5 years, BMI 26±2 kg/m², HbA1c 5.1±0.1 %, Rd 10±1 mg/kg·min) and 8 obese T2DM (4M/4F, age 50±3, BMI 36±2 kg/m², HbA1c 9±0.7 %, Rd 3±1 mg/kg·min) subjects using 2D gel-based proteomics in conjunction with pathway analysis to elucidate the mechanisms involved in the impairment of fatty acid oxidation. Insulin sensitivity was assessed using the euglycemic insulin clamp (80 mU/m² [bull] min) followed by vastus lateralis muscle biopsy. Twelve differentially expressed proteins obtained by PDQuest gel image analysis were identified by HPLC-ESI-tandem mass spectrometry and the differential expression was subsequently verified by immunoblotting. Four proteins were significantly over-expressed by ~ 30% ([Delta]^{3,5}-[Delta]^{2,4}-dienoyl-CoA isomerase, short-chain 3-hydroxyacyl-CoA dehydrogenase, enoyl-CoA hydratase and adenylate kinase 3; p<0.05) and 8 proteins were significantly under-expressed in T2DM as compared to NGT (by ~ 50%, p<0.05, pyruvate dehydrogenase protein component X, dihydrolipoamide S succinyltransferase, ATP synthase alpha-subunit, and mitofilin; by ~ 20%, p<0.05, [Delta]³-[Delta]²-enoyl-CoA isomerase, dihydrolipoamide dehydrogenase, cytochrome *c* oxidase subunit VIb, myosin regulatory light chain 2). Network and pathway analysis confirmed that the mitochondrial proteins with altered expression in T2DM participate in functions related to lipid metabolism and energy production. Membership in canonical pathways designated by Ingenuity Pathway Analysis was most enriched for lysine degradation (Ratio 4/136, p= 9.04 [acute] 10⁻⁸), fatty acid elongation in mitochondria (Ratio 3/45, p= 2.64 x 10⁻⁷) and fatty acid metabolism (Ratio 4/196, p= 2.84 x 10⁻⁶). Similarly, membership within the Gene Ontology Biological Process terms was most enriched for the categories of carboxylic acid metabolic process (p= 6.2 x 10⁻⁷) and fatty acid catabolic process (p= 3 x 10⁻⁴). More specifically, 6 of the enzymes are involved in the regulated production of acetyl CoA and intermediates of the tricarboxylic citric cycle, presumably from degradation of even-chain fatty acids and ketogenic amino acids. Mitochondrial dysregulation in human skeletal muscle could provide direct insights into the molecular pathogenesis of lipotoxicity in T2DM.

Nothing to Disclose: SK, AM, AOC, MAW, CAC, FC, DT, SEW, RAD, FF

Pub # P2-510
Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diabetes & Glucose Homeostasis: Genetic & Translational Approaches (1:30 PM-3:30 PM)
Title Interleukin-27 Gene Polymorphism (g.-964 A>G) in the 5[prime] Proximal Region Is Associated with Susceptibility to Type 1 Diabetes Mellitus in a Brazilian Cohort
Author String AS Santos, LG Crisostomo, ME Melo, RT Fukui, MER Silva
Laboratory of Medical Investigation-LIM 18 HC-FMUSP, São Paulo, Brazil

Body **Introduction**
Type 1 diabetes mellitus (DM1) results from the progressive and specific autoimmune destruction of insulin-secreting pancreatic beta-cells, elicited by lymphocytes and inflammatory cytokines. MHC is the major genetic factor underlying the development of diabetes in human and animal models but other susceptibility genes also contribute to the development of the disease. Upon activation, naive T helper cells are differentiated into TH1 or TH2 cells, depending on multiple influences by cytokines, types of antigen, transcription factors and signaling pathways. Besides the TH1 and TH2 lineages, a third population of T helper cells, producing IL-17 and designated TH17, had also been associated with autoimmunity. TH17 cells development is suppressed *in vitro* by another cytokine, the IL27 that plays a role in the development of the CD4 (+) T cell cytokine phenotype and can promote both anti and pro-inflammatory immune responses. IL-27 is a heterodimeric cytokine composed of EBI 3 and p28 subunits. An association with autoimmune diseases was observed for the SNP (g.-964A>G) at 5' proximal region of IL-27p28, but its relation to diabetes susceptibility had not been evaluated. **Objective:** To analyze the possible implication of the IL-27 polymorphism (g.-964A>G) in susceptibility to type 1 diabetes mellitus (DM1). **Patients and Methods:** Genomic DNA was extracted from 317 DM1 patients (age of diagnosis 19.6 ± 11.3 years, 130M/187F) and 295 healthy controls (130M/165F). The 5[acute]proximal region of the IL-27 p28 subunit encompassing 964bp nucleotides was amplified by PCR and submitted to sequencing or RFLP analysis. **Results:** The A and G allele frequencies were similar between patients and controls. However, there was an increased frequency of the heterozygote genotype in patients (60.2%) that exceeded significantly the frequency expected by the Hardy-Weinberg equilibrium ($p < 0.00005$). The controls sample did not show a significant departure from the equilibrium ($p = 0.55$) **Conclusion:** Our results suggest that the g.-964A > G polymorphism of IL-27p28 is associated with susceptibility to DM1A. This polymorphism within the binding site of the promoter region probably have an influence on the expression of *IL-27*, changing the affinity of binding between the transcription factor and its binding site, interfering with T cell responses.

Sources of Research Support: FAPESP.

Nothing to Disclose: ASS, LGC, MEM, RTF, MERS

Pub # P2-511

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diabetes & Glucose Homeostasis: Genetic & Translational Approaches (1:30 PM-3:30 PM)

Title Finger Printing Protein Patterns in Families and Their Generations with Diabetes Prevalence

Author String N Roohi, A Ahmad
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Body **Background and aims:** Implication of proteomics in early prognosis of type 1 and type 2 diabetes mellitus (T1 & T2 DM) and risk assessment of disease transmission within generations.
Subjects and Methods: We assessed serum protein profile of nine different families extending over three generations (grand parents to grand children) including diabetics, normal glucose tolerance (NGT) and impaired glucose tolerance (IGT) subjects. In these families, both T1 & T2 DM were prevalent. Three non-diabetic families with negative clinical and family history of diabetes were also included as a referent group. Protein fractions were resolved by SDS-PAGE, quantified, electroblotted, pertinent fractions expressing any relationship to the type of DM were excised out, electroeluted, run on IEF gels and identified by Swiss 2DPAGE database.
Results: Type 1 diabetics, in comparison with controls, exhibited highly elevated ($p=0.02$) C-reactive protein (CRP). Retinol binding protein (RBP) failed to express and transthyretin (TTR) was either significantly reduced ($p<0.001$) or totally unexpressed. In type 2 diabetics, CRP was more highly intensified ($p<0.0001$) even as compared to type 1 diabetics. RBP did not vary significantly ($p=0.55$) but TTR was either weakly expressed ($p=0.01$) or unexpressed. An unidentified fraction of 14.2 kDa manifested in all of the NGT subjects and in type 1 diabetics also failed to manifest in type 2 diabetics indicating its strong association with the development of T2 DM.
In diabetic families, some of the subjects with NGT who were non diabetic, symptomatically, and most IGT subjects exhibited deviated protein patterns closer to either T1 or T2 DM clearly reflecting their indisposition for ultimate development of T1 or T2 DM, respectively. One of the families was also having female twins in their third generation. One of the twins was clinically diagnosed type 2 diabetic, reflected also by her protein profile. The other member of the pair, although, non diabetic with NGT was strongly reflecting the protein pattern of her diabetic counterpart indicating her chances for the future development of T2 DM.
Conclusions: The specific trends of RBP and 14.2 kDa protein fractions due to their declining levels in IGT and failure of expression in type 1 and type 2 diabetics, respectively, may represent them as markers and predictors of pre-diabetic state and important targets in the treatment of pre-diabetes and prevention of type 1 and type 2 DM, respectively.

Nothing to Disclose: NR, AA

Pub # P2-512

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diabetes & Glucose Homeostasis: Genetic & Translational Approaches (1:30 PM-3:30 PM)

Title Insulin Action and Secretion in a Biracial Cohort with Low-Normal, High-Normal or Impaired Fasting Glucose

Author String E Chapp-Jumbo, C Edeoga, E Nyenwe, S Dagogo-Jack
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Body **Background:** Cross-sectional studies have shown a higher prevalence of type 2 diabetes mellitus (T2DM) and insulin resistance in African Americans (AA) than European Americans (EA). To determine the chronology of the ethnic disparities in glucoregulation, we studied a high-risk group (offspring of parents with T2DM) before the onset of diabetes.

Subjects and Methods: A total of 236 healthy offspring, mean (\pm SEM) age 46.4 ± 1.61 years, comprising 123 AA (88 women, 35 men) and 113 EA (75 women, 38 men) were enrolled. All subjects underwent a standard 75-g OGTT after an overnight fast, to document absence of diabetes. Based on the fasting plasma glucose (FPG) value, subjects were classified as Low-Normal Fasting Glucose (Low-NFG, <90 mg/dl), High-NFG (90-99 mg/dl) and Impaired Fasting Glucose (IFG, 100-125 mg/dl). Insulin sensitivity was assessed with hyperinsulinemic (2 mU/kg/min), euglycemic (100 mg/dl) clamp, and insulin secretion by calculating HOMA-B.

Results: The mean (\pm SEM) BMI (kg/m^2) was higher in AA than EA in the Low-NFG (30.3 ± 1.05 vs. 26.9 ± 1.26 , $P=0.041$), High-NFG (32.0 ± 1.06 vs. 28.2 ± 0.81 , $P=0.005$) and IFG (35.8 ± 1.30 vs. 29.2 ± 0.88 , $P=0.0001$) groups. The waist circumference (AA vs. EA) was 92.3 ± 2.36 vs. 85.8 ± 3.04 ($P=0.09$) in Low-NFG, 97.0 ± 2.43 vs. 92.3 ± 1.93 ($P=0.13$) in High-NFG, and 35.8 ± 1.30 vs. 29.2 ± 0.88 ($P=0.002$) in IFG subjects. Insulin sensitivity (Si-clamp, $\text{umol}/\text{kg}\cdot\text{min}^{-1}/\text{pmol}/\text{l}$) did not differ by race among subjects with Low NFG (AA 0.097 ± 0.01 vs. EA 0.105 ± 0.012 , $P=0.62$). Si-clamp decreased by 17% in the High-NFG group and by 30% in persons with IFG, compared with the Low-NFG group. The decline in insulin sensitivity was greater in AA than EA subjects, precipitously so among AA with IFG. The ethnic difference in Si-clamp was ~20% among persons with High-NFG (0.091 ± 0.006 vs. 0.073 ± 0.007 , $P=0.039$) and ~36% among those with IFG (0.084 ± 0.007 vs. 0.054 ± 0.006 , $P=0.003$). Insulin secretion was not significantly higher in AA than EA subjects in any subgroup.

Conclusion: In our biracial cohort of healthy offspring of parents with T2DM, AA subjects with FPG <90 mg/dl, despite having higher BMI, had similar insulin sensitivity compared to EA subjects with FPG <90 mg/dl. Compared to Low-NFG subjects, the occurrence of High-NFG and IFG is associated with increases in BMI, girth, and insulin resistance that were more marked in AA than EA. Compensatory insulin hypersecretion was not evident among the AA subgroups.

Dagogo-Jack S, Askari H, Tykodi G. Glucoregulatory Physiology in Subjects with Low-Normal, High-Normal, or Impaired Fasting Glucose. *J Clin Endocrinol Metab* 94:2031-2036, 2009

Sources of Research Support: National Institutes of Health (Grants R01 DK067269 and MO1 RR00211) and the American Diabetes Association.

Nothing to Disclose: EC-J, CE, EN, SD-J

Pub # P2-513

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diabetes & Glucose Homeostasis: Genetic & Translational Approaches (1:30 PM-3:30 PM)

Title Analysis of Beta-Cell Function and GLP-1 Levels in Carriers of Rs7903146 and Rs12255372 *TCF7L2* Genotypes in Mexican Pre-Diabetic Subjects

Author String LR Rocha-Ortiz, EL Perez-Luque, JM Malacara
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Body
Introduction. Genetic susceptibility to type 2 diabetes involves a number of variants, with a modest effect on the risk of diabetes. The *TCF7L2* gene encodes for a transcription factor that regulates proglucagon gene expression in enteroendocrine cells via Wnt signaling. Two polymorphisms of *TCF7L2* gene, rs12255372 and rs7903142 show robust association with type 2 diabetes.
Objective. To analyze the beta-cell function and GLP-1 levels in pre-diabetic subjects carries risk genotypes in *TCF7L2*.
Methods. We included 108 pre-diabetic subjects. Fasting blood samples was obtained to measure, glucose, lipid profile, insulin, C peptide, and GLP-1 (measured by RIA) levels and for DNA extraction. Insulin resistance and b-Cell function were calculated with HOMA model. The rs7903146C/T and rs12255372*TCF7L2* G/T polymorphisms were determined by PCR-RFLP. For analysis we made two groups: wild-type and carriers of one and two risk allele.
Results. We included to 24 men and 84 women with ages 45±11 year, BMI 33±6.5, serum glucose 109±6 mg/dl, Cholesterol 183±36 mg/dl; Triglycerides 162±98 mg/dl; LDL-Cholesterol 108±32 mg/dl; insulin 21.5±13 mIU/mL; C peptide 3.7±1.6 ng/mL; GLP-1 59±28 pMol/mL; HOMA-IR 5.8±3.4; HOMA-b-Cell 169±93. In carries rs7903146CT or TT, serum insulin, HOMA-IR, and HOMA-Beta-Cell were significantly lower than rs7903146CC genotype (p= 0.03; 0.04; and 0.02 respectively). In carries of rs12255372G/T or rs12255372 T/T also had lower levels of serum insulin, HOMA-IR, and HOMA-bCell (p=0.01; p=0.02; and p=0.004 respectively).
Conclusions. In the carries of one or two risk alleles of rs7903146C/T and rs12255372 *TCF7L2*G/T polymorphisms, serum insulin and HOMA-bCell function were significantly lower. This confirmed the influence of both *TCL7/L2* polymorphisms for susceptibility to T2DM.

Nothing to Disclose: LRR-O, ELP-L, JMM

Pub # P2-514

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diabetes & Glucose Homeostasis: Genetic & Translational Approaches (1:30 PM-3:30 PM)

Title Interaction between Bisphenol-A Exposure and a Genetic Variant in Insulin-Like Growth Factor 2 Receptor on Metabolic Phenotypes

Author String S Chanprasertyothin, L-o Chailurkit, B Ongphiphadhanakul
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Body Bisphenol-A (BPA), a monomeric component of polycarbonate plastic, is an endocrine disruptor. Besides its relationship with sexual development, recent studies have demonstrated the association between BPA exposure and metabolic phenotypes. BPA influences insulin-like growth factor-1 (IGF1) and IGF1 is involved in many biological processes including glucose metabolism. In the present study, we examined the probable interaction between BPA exposure and genetic variant in insulin-like growth factor-2 receptor (IGF2), a receptor playing a role in the degradation of IGF1. Subjects consisted of 240 individuals aged at least 50 years randomly selected within each glucose tolerance status from an oral glucose tolerance study of 661 subjects. Eighty of the subjects had normal glucose tolerance while 80 had impaired glucose tolerance and 80 had diabetes. Serum BPA was measured by ELISA (IBL International GmbH, Germany). A rs416572 single nucleotide polymorphism (SNP) of insulin-like growth factor 2 receptor was genotyped by using real-time PCR (TaqMan^{reg} MGB probes). Data were expressed as mean \pm SE. Of all the 240 subjects, 132 (55%) had detectable serum BPA (BPA+). The distribution of IGF2R genotypes based on SNP rs416572 were 70.4% CC, 27.5% CT and 2.1%TT. Analysis of variance revealed a statistical interaction between BPA exposure and IGF2R genotype in determining a number of metabolic phenotypes including body mass index (BMI) ($P < 0.05$) and HDL-cholesterol (HDL-C) ($P < 0.05$). In subjects negative for BPA exposure, the presence of the rs416572 T allele was associated with lower BMI (24.8 \pm 0.7 vs. 26.7 \pm 0.5 $P < 0.05$), lower 2-hr plasma glucose after oral glucose (153.5 \pm 13.0 vs. 190.5 \pm 8.6 $P < 0.05$) and HDL-C (43.7 \pm 2.5 vs. 35.6 \pm 1.6 $P < 0.05$) independent of age and gender. On the contrary, in subjects positive for BPA exposure, no association between IGF2R genotype and metabolic phenotypes was found. Conclusions BPA exposure modifies the genetic influence of IGF2R on metabolic phenotypes.

Sources of Research Support: Mahidol University fund.

Nothing to Disclose: SC, L-OC, BO

Pub # P2-515

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diabetes & Glucose Homeostasis: Genetic & Translational Approaches (1:30 PM-3:30 PM)

Title Ethnic Differences in Insulin-Stimulated Glucose Disposal, Hepatic Insulin Sensitivity, and Endogenous Glucose Production among African-American and European-American Women

Author String A Ellis, J Alvarez, W Granger, F Ovalle, B Gower
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Body **Background:** Intravenous glucose tolerance tests (IVGTT) have demonstrated lower whole-body insulin sensitivity (S_I) among African Americans (AA) compared to European Americans (EA). However, whole-body S_I represents a composite of both insulin-stimulated glucose disposal, which occurs primarily at skeletal muscle, and sensitivity to insulin's suppression of endogenous glucose production, which occurs at the liver. A mathematical model was recently introduced that now allows for distinction between disposal and hepatic insulin sensitivity by IVGTT. The purpose of this study was to examine specific indexes of insulin sensitivity among healthy, premenopausal AA and EA women to determine whether lower whole-body insulin sensitivity among AA may be attributed to insulin action at skeletal muscle, liver, or both.

Methods: Participants were 53 non-diabetic, premenopausal AA and EA women. Profiles of endogenous glucose production and specific indexes of Disposal S_I and Hepatic S_I were calculated by incorporating a stable isotope tracer ($6,6\text{-}^2\text{H}_2$ glucose) into the IVGTT and applying a new 2-compartment mathematical model (1). Body composition was assessed by dual energy X-ray absorptiometry.

Results: Both Disposal S_I and Hepatic S_I were lower among AA ($p<0.05$) independent of body composition. Hepatic S_I accounted for 1/3 total insulin sensitivity for both EA and AA. Time profiles for serum insulin and endogenous glucose production (EGP) revealed higher peak insulin response to glucose and lower EGP among AA women compared to EA.

Discussion: Calculated indexes from a recently-introduced mathematical model suggest that lower whole-body insulin sensitivity among non-diabetic AA women is due to both hepatic and peripheral components. Yet, despite lower Hepatic S_I , AA displayed lower EGP. Future research is needed to determine the physiological basis of lower EGP among AA, and its implications for type 2 diabetes risk.

(1) Tokuyama K et al., Diabetes Technology and Therapeutics 2009;11:487-492

Sources of Research Support: NIH Grants R01DK58278; M01-RR-00032; UL 1RR025777; P30-DK56336; P60DK07962; F31 AT005384-01.

Nothing to Disclose: AE, JA, WG, FO, BG

Pub # P2-516

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diabetes & Glucose Homeostasis: Genetic & Translational Approaches (1:30 PM-3:30 PM)

Title Metabolic Effects of Whole Body Vibration Training in Latino Boys

Author String DN Erceg, LJ Anderson, CM Nickles, CJ Lane, ET Schroeder
University of Southern California, Los Angeles, CA

Body **PURPOSE:** With an increasing obesity epidemic, efficient methods of exercise are sought to improve health. Vibration training has been in use for decades to improve strength and flexibility, however little is understood about the metabolic effects from whole body vibration (WBV) exercise. We sought to determine if WBV exercise can improve insulin/glucose dynamics, glycosylated hemoglobin (HbA_{1c}), and lipid profile in sedentary overweight Latino boys. **METHODS:** 20 overweight prepubertal Latino boys 8-10 yrs of age were randomly assigned to either a control (CON = 9) or 3 days per week WBV exercise (VIB = 11) for 10 weeks. Changes in metabolic parameters were assessed at baseline and 48 hours post last training session. Glucose/insulin indices were determined through an oral glucose tolerance test. Comparisons of changes within and between groups for variables of interest were conducted using *t* tests and general linear model ($\alpha < 0.05$). **RESULTS:** There was a significant decrease in fasting glucose ($p=0.04$), triglycerides ($p=0.02$), and VLDL cholesterol ($p=0.02$) for the CON group. Between groups analyses revealed the CON group significantly lowered VLDL cholesterol ($p=0.02$) and triglycerides ($p=0.02$) compared to the WBV exercise group. The WBV program did not result in statistically significant improvements in fasting and 2hr insulin, glucose, insulin resistance index (HOMA-IR), insulogenic index, HbA_{1c} or lipid profile. **CONCLUSION:** A controlled 10 wk WBV exercise program may not be effective for altering fasting insulin and glucose, insulin resistance, HbA_{1c} or lipids in prepubertal Latino boys.

Nothing to Disclose: DNE, LJA, CMN, CJL, ETS

Pub # P2-517

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diabetes & Glucose Homeostasis: Genetic & Translational Approaches (1:30 PM-3:30 PM)

Title Activation of Heat Shock Response Reduces Visceral Adiposity, Improves Insulin Resistance and Inflammatory Markers in Male Subjects with Metabolic Syndrome and Type 2 Diabetes

Author String T Kondo, R Matsuyama, S Koga, K Miyagawa, R Goto, MA Suico, J Kawashima, H Motoshima, N Furukawa, K Tsuruzoe, H Kai, E Araki
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Body Reduction of heat shock protein (Hsp) 72 is associated with insulin resistance and chronic inflammation, because Hsp72 is regulated by GSK-3 β and is also a natural inhibitor of JNK and ASK1. Induction of Hsp72 improves glucose intolerance and insulin sensitivity, and reduces visceral adiposity in diabetic model mice (1). However, its impact on human with metabolic syndrome (MS) or type 2 diabetes (T2DM) is unknown. Forty male subjects with MS or 40 of those with T2DM were randomly assigned into 2 groups, respectively. One group was treated with MET (Mild Electrical stimulation with Thermo: 12V, 55 pulses per second, 60 min at 42oC, 4 times a week) for 12 weeks followed by 12 weeks of non-treatment. The order was reversed in the other group.

MET treatment did not induce any unfavorable effects nor muscle contraction/pain. In MS subjects, visceral adipose volume measured by abdominal CT scan was significantly decreased by $5.79 \pm 0.36\%$ ($p=0.00079$) and waist circumference was also reduced by $0.70 \pm 0.08\%$ ($p=0.017$), whereas body weight and subcutaneous adipose volume were not. Both systolic and diastolic blood pressure were significantly reduced by $4.19 \pm 0.27\%$ ($p=0.0039$) and $2.7 \pm 0.28\%$ ($p=0.033$), respectively. Fasting plasma glucose and insulin levels were both decreased by $4.87 \pm 0.33\%$ ($p=0.0035$) and $8.49 \pm 0.91\%$ ($p=0.015$), respectively. HbA1c showed a trend of reduction, but could not reach statistical significance ($-1.19 \pm 0.14\%$, $p=0.065$). HOMA-IR, QUICKI and composite WBISI were all significantly improved by $-11.7 \pm 1.0\%$ ($p=0.0048$), $+2.44 \pm 0.18\%$ ($p=0.0046$) and $+17.2 \pm 1.0\%$ ($p=0.0014$), respectively, while insulinogenic index (I.I.) was significantly decreased by $10.2 \pm 2.0\%$ ($p=0.041$). Inflammatory cytokines or adipokines, such as hs-CRP, adiponectin, leptin and TNF- α were all improved by $-27.4 \pm 5.9\%$ ($p=0.028$), $+9.3 \pm 1.1\%$ ($p=0.035$), $-8.27 \pm 0.87\%$ ($p=0.022$) and $-10.22 \pm 1.00\%$ ($p=0.044$), respectively. In addition, WBC and LDL-C were decreased by $5.38 \pm 0.56\%$ ($p=0.028$) and $5.26 \pm 0.37\%$ ($p=0.005$), respectively. An interim report of T2DM trial indicated similar results as in MS so far.

MET treatment improved glucose homeostasis and insulin resistance in males with MS or T2DM. Reduction of insulin secreting capacity in MS suggested by reduced I.I. may protect pancreatic β -cells from overproduction of insulin. In addition, significant reductions in inflammatory cytokines, WBC and LDL-C suggest that MET also prevent atherogenic complications.

(1) Morino S, Kondo T et al., PLoS ONE 2008; 3(12): e4068

Nothing to Disclose: TK, RM, SK, KM, RG, MAS, JK, HM, NF, KT, HK, EA

Pub # P2-518

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diabetes & Glucose Homeostasis: Genetic & Translational Approaches (1:30 PM-3:30 PM)

Title Insulin Signaling Paradox Characterizes Human Insulin Resistance

Author String KT Tonks, Y Ng, S Miller, ACF Coster, D Samocha-Bonet, TJ Iseli, A Xu, DJ Chisholm, DE James, JR Greenfield
Garvan Institute of Medical Research, Sydney, Australia; University of New South Wales, Sydney, Australia; University of Hong Kong, Hong Kong, Hong Kong

Body

Background and Aims: Impaired insulin-mediated glucose uptake into muscle contributes to several metabolic disorders, including type 2 diabetes. It is proposed that this impairment involves a defect in proximal elements of the PI3K/Akt signalling pathway, which would result in reduced insulin-dependent phosphorylation of Akt and its substrates in insulin-resistant individuals.

Methods: To test this hypothesis, vastus lateralis muscle biopsies were taken before and during a hyperinsulinemic-euglycemic clamp from 81 human subjects spanning a wide range of adiposity and insulin sensitivity. We included an overweight/obese insulin-sensitive group, allowing us to dissect effects of adiposity from insulin resistance.

Results: Insulin-stimulated phosphorylation of Akt at Thr309 and Ser474, a common surrogate of Akt activity, correlated strongly with insulin sensitivity in the whole cohort. However, there were no significant relationships between insulin-stimulated phosphorylation of Foxo, PRAS40 or AS160 and insulin sensitivity and insulin-stimulated Akt phosphorylation. Insulin-stimulated AS160 phosphorylation was more closely related to subcutaneous adipose tissue than with insulin sensitivity. Multivariate regression analysis revealed that 50% of the variance in whole body insulin sensitivity was explained by insulin-stimulated muscle Akt phosphorylation at Thr309, fasting adiponectin and FGF21 levels and circulating fatty acid levels during the clamp.

Conclusion: This study suggests that Akt Thr309 and Ser474 phosphorylation is a poor indicator of downstream *in vivo* Akt activity. We conclude that impaired Akt activity in skeletal muscle may not be the critical mediator of human insulin resistance.

Nothing to Disclose: KTT, YN, SM, ACFC, DS-B, TJI, AX, DJC, DEJ, JRG

Pub # P2-519

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diabetes & Glucose Homeostasis: Genetic & Translational Approaches (1:30 PM-3:30 PM)

Title A Low-Dose Insulin Infusion Suppresses the Expression of β -Amyloid Precursor Protein

Author String P Dandona, I Mohamed, H Ghanim, CL Sia, S Dhindsa, A Makdissi, A Chaudhuri
State University of New York at Buffalo, Buffalo, NY

Body Since Alzheimer's disease (AD) is characterized by chronic inflammation in the brain and since the major pathognomonic lesions of this disease are the amyloid plaque and the neurofibrillary tangle and since a low dose infusion of insulin exerts an anti-inflammatory effect, we hypothesized that insulin may suppress the expression of the proteins involved in the formation of these lesions. The plaque is formed by the β -amyloid which is formed from amyloid Precursor Protein (APP) by the enzymes, β -secretase and γ -secretase which have presenilin-1 (PS-1) and presenilin-2 (PS-2) subunits. The neuro-fibrillary tangle is formed from tau protein following its hyperphosphorylation by the enzyme GSK-3 β . Ten fasting patients with type 2 diabetes were infused with insulin (2U/h) with glucose (5g/h) for 4h. Blood samples were collected at 0, 2, 4 and 6h. Peripheral blood mononuclear cells (MNC) and plasma were prepared for analysis. There was a significant reduction in the expression of APP, PS-1, PS-2 and GSK-3 β by 19 \pm 5, 24 \pm 9, 28 \pm 6 and 40 \pm 7% respectively (p <0.05, for all) in MNC. This occurred in parallel with reductions in ROS generation, intranuclear NF κ B binding and the expression of pro-inflammatory cytokines in MNC (p <0.05 for all). There was a significant reduction in plasma concentration of serum amyloid A and intercellular adhesion molecule-1 (p <0.05 for both). We conclude that insulin induces a rapid suppression of APP, PS-1, PS-2 and GSK-3 β , all of which are cardinal in the pathogenesis of AD. This suppression occurs in parallel with other comprehensive anti-inflammatory effects. Insulin may have a potential in the treatment of AD provided it can be delivered into the brain directly avoiding its hypoglycemic effects. Indeed, it is currently being tried in the treatment of AD through intranasal delivery.

Sources of Research Support: NIH Grants R01-DK075877 and R01-DK069805 awarded to PD; American diabetes Grant awarded to PD; American Diabetes Grant 10-JF-13 awarded to SD.

Disclosures: PD: Principal Investigator, GlaxoSmithKline; Clinical Researcher, Sanofi-Aventis; Speaker, Novartis Pharmaceuticals; Eli Lilly & Company; GlaxoSmithKline; Sanofi-Aventis. SD: Speaker, Abbott Laboratories. AC: Speaker, Eli Lilly & Company. Nothing to Disclose: IM, HG, CLS, AM

Pub # P2-520

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diabetes & Glucose Homeostasis: Genetic & Translational Approaches (1:30 PM-3:30 PM)

Title The Effect of Work Intensity and Hypoxia on Glucose and Insulin Sensitivity in Type 2 Diabetics

Author String R Mackenzie, N Maxwell, B Elliott, G Brickley, P Watt
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Body Hypoxia and muscle contraction stimulate glucose transport activity in vitro[1]. We previously demonstrated exercise and hypoxia have a similar effect on insulin sensitivity in type 2 diabetics and when performed together, the effects were additive [2]. If higher intensity of exercise over shorter amounts of time could produce similar results, exercise adherence would be expected to improve. The aim of this study was therefore to examine whether total work or exercise intensity had a greater influence on glucose metabolism following acute hypoxic exercise in type 2 diabetics. Methods: Eight male type 2 diabetics completed 60 minutes of hypoxic [$PO_2 \approx 14.7$ (0.2)%] exercise at 90% of lactate threshold [Hy Ex60; 49 (1) Watts]. Patients completed a further two hypoxic trials of equal work, lasting 40min [Hy Ex40; 70 (1) Watts] and 20min [Hy Ex20; 140 (12) Watts]. Glucose Ra and Rd were determined using an adapted non-steady-state one-compartment model. Homeostasis (HOMA) models of insulin sensitivity were calculated for the 24 and 48hr post hypoxic exercise Results: Glucose disposal was acutely elevated in Hy Ex60 ($P = 0.001$) and Hy Ex40 ($P = 0.005$) but not Hy Ex20 ($P = 0.118$). Fasting blood glucose concentrations were reduced in Hy Ex60 ($P=0.48$) and Hy Ex40 ($P=0.02$) but not Hy Ex20 (0.13) 48 hours post exercise. Both Ra and Rd was acutely increased at several time points in Hy Ex20 ($P<0.05$) but not Hy Ex60 or Hy Ex 40 ($P<0.05$). 24 and 48 hours post exercise both Hy Ex60 and Hy Ex40 showed trends towards improvements, while Hy Ex20 did not. QUIKI demonstrated improvements in both Hy Ex60 and Hy Ex40 post exercise ($P<0.05$ at all time points). QUIKI showed no changes in Hy Ex20. Conclusions: Moderate-intensity exercise in hypoxia (Hy Ex60 and Hy Ex40) stimulates and moderate-term improvements in insulin sensitivity that was less apparent in Hy Ex20. Results suggest that exercise duration and not total work performed has a greater influence on acute and moderate-term glucose tolerance in type 2 diabetics. Application of these findings in clinical settings could reduce the exercise load needed to produce positive effects in the treatment of type 2 diabetics, increase adherence to exercise treatments and potentially reduce the rate of pharmaceutical intervention in these populations.

(1) Fisher, J.S., et al., Activation of AMP kinase enhances sensitivity of muscle glucose transport to insulin. *Am J Physiol Endocrinol Metab*, 2002. 282(1): p. E18-23.

(2) Mackenzie, R., et al., Acute hypoxia and exercise improve insulin sensitivity (S(I) (2) *) in individuals with type 2 diabetes. *Diabetes Metab Res Rev*. 27(1): p. 94-101.

Nothing to Disclose: RM, NM, BE, GB, PW

Pub # P2-521

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diabetes & Glucose Homeostasis: Genetic & Translational Approaches (1:30 PM-3:30 PM)

Title Transcriptional Activator MEF2A Is Overexpressed in Adipocytes of PCOS Patients

Author String Y-H Chen, S Heneidi, R Azziz
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Body Glucose transporter type 4 (GLUT4) expression strongly influences the development of insulin resistance. Depletion of GLUT4 in adipose tissue increases insulin resistance in both muscle and liver. Compared to healthy control (1.59 ± 1.04 , $n=8$; mean \pm SD, arbitrary unit) in adipocytes, our data indicates GLUT4 protein expression is reduced in insulin resistant individuals (0.24 ± 0.17 , $n=7$, $p<0.01$) and patients with PCOS (0.19 ± 0.15 , $n=16$, $p<0.01$). This reduction of GLUT4 expression is due to a decrease in gene transcription. The myocyte enhancer factor 2 (MEF2) family transcription factor (MEF2A and MEF2D) and GLUT4 enhancer factor (GEF) have been identified to regulate GLUT4 gene promoter activity in vitro and in vivo. In vitro, both MEF2A and GEF are needed in order to active promoter activity, whereas MEF2D inhibits transcription activity. Following our GLUT4 expression data, we expected MEF2A would be decreased in those with insulin resistance or PCOS. However, an increase of MEF2A protein is observed in individuals with insulin resistance and PCOS (healthy controls: 0.135 ± 0.136 , $n=6$; insulin resistant individuals: 1.06 ± 0.95 , $n=6$, $p<0.05$ vs healthy control; PCOS: 0.86 ± 0.67 , $n=10$, $p<0.05$ vs healthy control), and this increase is not due to transcription activity. An increase of MEF2A suggests MEF2A is not the factor that reduces GLUT4 gene transcription activity, but the result of a GLUT4 reduction feedback effect. GLUT4 is a potential therapeutic target for the treatment of insulin resistance. The factors that inhibit GLUT4 expression in adipocytes of insulin resistance or PCOS are still unclear and need to be determined.

Nothing to Disclose: Y-HC, SH, RA

Pub # P2-522

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diabetes & Glucose Homeostasis: Genetic & Translational Approaches (1:30 PM-3:30 PM)

Title Regulation of RBP4 Effect on Insulin Response by STRA6 in Human Adipocyte

Author String J-G Chen, A Spagnoli, Z Pamuklar, A Torquati
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Body Adipose-derived retinol-binding protein 4 (RBP4) has been shown to contribute to insulin resistance in mice. However inconsistent data exists for the role of RBP4 in humans. STRA6 (*stimulated by retinoic acid*) has been established as a high-affinity cell-surface receptor for RBP4 and we previously reported that STRA6 expression in subcutaneous adipose tissue was correlated with insulin resistance in humans (Chen et al, 2010, Endocrine Society annual meeting). We hypothesize that STRA6 acts as downstream mediator of RBP4 to regulate its effect on insulin signaling.

Adipose tissues obtained from: 1) lean subjects (n=16) undergoing elective abdominal surgery, 2) obese subjects (n=21) with or 3) without diabetes (n=20) undergoing gastric bypass surgery and were subjected to quantitative real time PCR. Human subcutaneous adipose stem cells derived from lean and nondiabetic donor were induced to differentiation into adipocytes with established protocol.

We found that STRA6 expression level in subcutaneous adipose tissues was up-regulated 2.7 folds in obese subjects with diabetes compared with lean subjects (p<0.05). Insulin signaling was down-regulated STRA6 expression while acute exposure to hTNF α induced STRA6 mRNA levels in differentiated adipocytes. Furthermore, in adipocytes, recombinant hRBP4 in time-dependent manner impaired insulin-stimulated phosphorylated-Akt activity and glucose uptake was significantly inhibited in RBP4 treated adipocytes. In contrast, insulin-stimulated Akt phosphorylation was unaffected by RBP4 exposure in STRA6 knockdown adipocytes. Consistently, RBP4 exposure did not significantly alter insulin-stimulated glucose uptake in STRA6 knockdown adipocytes.

Our results show that STRA6 acts as downstream of RBP4 and is required for RBP4 to induce insulin resistance in human adipocytes. These findings imply that STRA6 may play an important role in the development of obesity-derived insulin resistance.

Sources of Research Support: This work was supported by National Institutes of Health K23DK075907(to T.A.).

Nothing to Disclose: J-GC, AS, ZP, AT

Pub # P2-523

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diabetes & Glucose Homeostasis: Genetic & Translational Approaches (1:30 PM-3:30 PM)

Title Carbohydrate Supplementation Attenuates Cell-Free Plasma DNA Increase in Elite Runners Undergoing Intensive Training: Hormonal Interactions

Author String MV Sousa, K Madsen, R Fukui, A Santos, MER Silva
São Paulo University, São Paulo, Brazil; Aarhus University, Aarhus, Denmark

Body Introduction: A comprehensive training program combined with an inadequate rest periods and an inappropriate diet designed to aid muscle recovery can result in overtraining syndrome. This is linked with increased susceptibility to infections due to changes in the functional status of immune cells and biochemical abnormalities, with consequent damage to athletic performance (1). A number of studies have linked markers for these changes, such as free plasma DNA, to athletes' recovery (2).
Objective: To evaluate the effect of carbohydrate supplementation on cell-free plasma DNA and conventional indices of overtraining for long distance runners undergoing overload training.
Method: Twenty-four male elite runners (28.0±1.2 years) were randomly assigned to 2 groups (Carbohydrate: CHO-group and Control: CON-group) using a double-blind design. Athletes from both groups took part in overload training for 8 days followed by a high intensity intermittent running protocol (10x800m) on the 9th day. The CHO-group ingested maltodextrin as a carbohydrate solution during every training session while the CON-group was supplied with a placebo solution.
Results: After 8 days of intensive training, LDH baseline levels remained constant in the CHO-group (from 449.1±18.2 to 474.3±22.8 U/L) and increased in the CON-group (from 413.5±23.0 to 501.8±24.1 U/L, p<0.05). On the 9th day LDH concentrations were lower in the CHO-group (509.2±23.1 U/L) than in the CON-group (643.3±32.9 U/L, p<0.01) immediately post 10x800m. CHO ingestion attenuated the increase of free plasma DNA post 10x800m (48240.3±5431.8 Allels/ml) when compared to the CON group (73751.8±11546.6 Allels/ml, p<0.01). Leukocyte counts were attenuated in the CHO group (9.1±0.1 cells/[micro]L) following 10x800m when compared with the CON group (12.2±0.7 cells/[micro]L; p<0.01) and at the 80 min of the recovery period (CHO: 10.6±0.1 cells/[micro]L vs. CON: 13.9±1.1 cells/[micro]L; p<0.01). Cortisol levels were correlated positively with free plasma DNA, leukocytes and LDH (r>0.40, p<0.001). Adrenaline levels were correlated only with free plasma DNA (r>0.40, p<0.001).
Conclusion: Our findings showed for the first time that ingestion of a carbohydrate beverage resulted in less DNA damage post-exercise so providing a better recovery during intensive training.
Key-words: free plasma DNA, tissue damage, inflammation, overtraining, intensive training.

(1) Angeli A et al., J Endocrinol Invest 27: 603-612, 2004.
(2) Fatouros IG et al., Clinical Chemistry 52: 1820-21, 2006.

Sources of Research Support: FAPESP.

Nothing to Disclose: MVS, KM, RF, AS, MERS

Pub # P2-524

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diabetes & Glucose Homeostasis: Genetic & Translational Approaches (1:30 PM-3:30 PM)

Title A Virtual Patient Model for Comparing, Adapting and Designing ICU Insulin Infusion Protocols

Author String BG Buss, JW Grizzle, JM Blum, RY Gianchandani
University of Michigan Medical Center, Ann Arbor, MI; University of Michigan, Ann Arbor, MI; University of Michigan, Ann Arbor, MI

Body Glucose control in the ICU is known to improve morbidity and mortality. However, as glucose targets evolve, ICUs need to update their respective protocols. Safety remains key. To permit the systematic evaluation, design and modification of insulin infusion protocols, a virtual patient model has been developed from ICU patient data collected roughly hourly. We demonstrate the use of the model for predicting differences in key outcomes resulting from existing protocols; a similar process would be used to design or modify a protocol to meet new requirements.

The model contains a *deterministic* component that represents expected physiological behavior and reflects insulin sensitivity, insulin production and other individual factors. Unlike several other models (1,2), it also has a *stochastic* (or random) component, which includes a statistical description of glucose variability due to medications, (esp. vasopressors and steroids), nutrition, changing clinical condition, etc. This component makes the model more suitable for predicting the performance of an insulin protocol (3). We separately applied insulin infusion protocols of the University of Michigan (UM), University of North Carolina (NC), Yale-New Haven (NH), and a Glucomander-like protocol (GL) to the virtual patient model. The model was used to simulate glucose trajectories over a 48 hour period for each of the 196 patients used in the construction of the model. We calculated the percent of simulated BG measurements indicating hypoglycemia (BG<70 mg/dl) and severe hypoglycemia (<40 mg/dl); time to reach the goal range 100-140 mg/dl; time in goal; and glycemic coefficient of variation (CV). CV is standard deviation divided by the mean. The results are given below, together with the values for the actual patients (AP).
Summary of simulation results:
Clinical Outcomes: UM/ /NC/ /NH/ /GL/ /AP
Hypoglycem. (%) 1.12 2.54 2.96 0.93 0.29
Severe hypo (%) 0.02 0.07 0.30 0.04 0.00
Time to goal (hr) 1.78 1.55 1.62 2.26 4.00
Time in goal (%) 60.1 59.5 54.2 63.9 59.6
Glycemic CV (%) 18.2 21.3 25.6 19.5 20.5

Our aim is to construct a public domain model into which hospitals can input their insulin protocol and benchmark it for the above outcomes before patient testing. On the basis of collected ICU data, informed changes and adjustments can be made. Therefore any institution could use this model to improve its protocol's performance and respond to changing glucose targets as established norms evolve.

(1) Hovorka R et al. *Physiol Meas* 2008; 29:959.
(2) Van Herpe T et al. *J Diabetes Sci Technol* 2007; 1:348.
(3) Lin J et al. *Biomed Signal Process Control* 2006; 1:229.

Sources of Research Support: NSF EAGER grant 0938288.

Nothing to Disclose: BGB, JWG, JMB, RYG

Pub # P2-525

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diabetes & Glucose Homeostasis: Genetic & Translational Approaches (1:30 PM-3:30 PM)

Title Pioglitazone Inhibits TNF- α Promoter Transcriptional Activity in Human Mesangial Cells

Author String FA Milton, TdO Menezes, MAD Costa, AA Amato, M Togashi, FdAR Neves
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Body INTRODUCTION: Mesangial cells (MC) regulate glomerular filtration, and are involved in the pathogenesis of many glomerular diseases including diabetic nephropathy. It is known that the tumor necrosis factor-alpha (TNF-alpha) is involved in the pathogenesis of renal diseases and particularly in the proliferation of MC. Thiazolidinediones (TZDs), drugs used for treatment of diabetes mellitus, improves insulin resistance and are effective in preventing the progression of renal disease in various experimental models including diabetic nephropathy. This protection seems to be secondary to the anti-inflammatory effect of TZDs. Given that TNF alpha is a potent inflammatory agent and is produced by mesangial cells, the goal of this work is to investigate whether pioglitazone (PIO), a TZD, reduces proliferation of CM induced by TNF-alpha, and also whether this effect is mediated by transcriptional repression of TNF-alpha promoter.

METHODS: CM immortalized human and U937 cells grown were treated with TNF-alpha or vehicle in combination or not with PIO (10-5M). The incorporation of 3H-thymidine was used to assess pa mesangial proliferation. To measure promoter activity of TNF-alpha, we used luciferase reporter gene. In this essay we co-transfected an expression vector luciferase gene driven by the promoter of TNF- α with the following constructs: -1044 / +93 TNF-Luc, which contains 1044 bp of the promoter of TNF-alpha; -125 TNF-Luc plasmid and plasmid-TNFRE3 TK Luc, contains three copies of the portion -125/-82. In some experiments also co-transfected the expression vector of the peroxisome proliferator receptor gamma (PPAR γ).

Results: PIO decreased the proliferation of MC to 61.1 + 4.4% compared to those treated with vehicle. TNF- α in turn, increased significantly (216.8 + 20.6%) the MC proliferation and co-treatment with TNF-alpha and PIO decreased it to baseline levels (112 + 13.4%). When co-transfected with PPAR γ the addition of PIO significantly reduced the activity of TNF-alpha promoter to 73.5% +3.8 and 70.1 +2.3% in the absence and presence of TNF-alpha , Respectively (p <0.001 vs PIO EtOH). This action of PPAR γ uses preferably the region between -125/-82 regions of the promoter and has an IC50 of 1.7 x 10-7 M. Unlike the MC this repression effect was not observed in human U937 monocytes.

Conclusions: These results suggest that part of the therapeutic effect of TZDS on mesangial cells are mediate by transcriptional repression of TNF-alpha promoter by PPAR γ .

Nothing to Disclose: FAM, TdOM, MADC, AAA, MT, FdARN

Pub # P2-526
Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diabetes & Glucose Homeostasis: Genetic & Translational Approaches (1:30 PM-3:30 PM)
Title Finger-Stick Glucose Values Are an Essential Complement to Hemoglobin A1c and Fructosamine in HIV-Infected Patients with Diabetes
Author String S-Y Kim, P Friedmann, A Seth, AM Fleckman
Beth Israel Medical Center, New York, NY; Beth Israel Medical Center, New York, NY; Beth Israel Medical Center, New York, NY

Body

Introduction

Diabetes mellitus (DM) in HIV patients is 4.6 times more prevalent than in the general population.¹ Current DM management in HIV patients follows general guidelines.² However, published studies report inappropriately low hemoglobin A1C values (A1C), underestimating glycemia in HIV patients.³

Objective

To determine if the reported underestimation of A1C in predicting glycemia in HIV DM is consistent with the findings in our patients.

Methods

We reviewed the charts of 65 HIV DM patients at our clinic; 59 had available data. We extracted the most recent A1C; fructosamine and finger stick glucose (FS) averaged over 3 months; and the number of FS during that time. Predicted average glucose (AG) was calculated as reported by Nathan et al⁴: $AG \text{ in mg/dL} = 28.7 \times A1C - 46.7$, which estimates a change of 29 mg/dL for each 1% change in A1C, a magnitude we determined to be clinically relevant. Data were analyzed using the SAS system to assess the correlation between fructosamine, A1C and AG. We also examined the proportion of patients whose predicted and actual AG values differed by more than 29 mg/dL.

Results

Of the 59 patients examined, 15% had A1C values underestimating AG by more than 29 mg/dL, while 25% overestimated AG by more than 29 mg/dL. For the remaining 60%, A1C estimated AG within the established range. Given the variability in number of available FS, we further examined results for patients who had at least 7 FS values (n=23). For this subgroup, A1C underestimated AG in 22% of the cases and overestimated it in 17%. A Pearson correlation coefficient was computed between A1C and AG ($r=0.55$, $p<0.0001$) and between fructosamine and AG ($r=0.37$, $p=0.0688$). Our findings suggest a moderate correlation between A1C and AG and a poor correlation between fructosamine and AG. Our analysis also revealed that A1C under and overestimates FSG values in 40% of our HIV DM patients.

Conclusions

We found a stronger correlation between A1C and AG than between fructosamine and AG. Both correlations were weaker than expected. We also found variability in the direction of the discrepancy between A1C and AG. Our data bring into question the current reliance on A1C and fructosamine among HIV DM patients. Until further studies become available, these findings suggest that A1C may have greater utility than fructosamine in predicting AG in HIV DM, and emphasize the importance of complementing A1C and fructosamine values with accurate FS reporting in this patient population.

- (1) Diop ME, Bastard JP, Meunier N, Thevenet S, Maachi M, Capeau K, Pialous G, Vigouroux C. Inappropriately Low Glycated Hemoglobin Values and Hemolysis in HIV-Infected Patients. *AIDS Res Hum Retroviruses* 2006;22:1242-1247
- (2) Lundgren JD, Bategay M, Behrens G, De Wit S, Guaraldi G, Katlama C, Martinez E, Nair D, Powderly WG, Reiss P, Sutinen J, Vignano A. European AIDS Clinical Society (EACS) guidelines on the prevention and management of metabolic diseases in HIV. *HIV MED* 2008;9:72-81
- (3) Kim PS, Woods C, Georgoff P, Crum D, Rosenberg A, Smith M, Hadigan C. A1C Underestimates Glycemia in HIV Infection. *Diabetes Care* 2009;32:1591-1593
- (4) Nathan DM, Kuenen J, Borg R, Kuenen J, Borg R, Zheng H, Schoenfeld D, Heine RJ. Translating the A1C Assay Into Estimated Average Glucose Values. *Diabetes Care* 2008;31:1-6

Nothing to Disclose: S-YK, PF, AS, AMF

Pub # P2-527

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diabetes & Glucose Homeostasis: Genetic & Translational Approaches (1:30 PM-3:30 PM)

Title Automatic Snacking Prevents Hypoglycemia among Admitted Type 2 Diabetic Patients on Intensive Insulin Therapy: A Single-Blinded Randomized Controlled Trial

Author String ZG Lorenzo, NER Laplano, LB Mercado-Asis
University of Santo Tomas Hospital, Manila, Philippines

Body Context: Acute hyperglycemic crisis is seen among critically ill admitted type 2 diabetic patients warranting intensive insulin therapy. However, the major drawback of such treatment is hypoglycemia, which may lead to adverse cardiovascular outcomes and even death. Therefore, we designed a scheme to overcome this complication through automatic snacking.
Objective: The aim is to demonstrate that automatic snacking prevents hypoglycemia among admitted diabetic patients on intensive insulin therapy.
Methods: Twenty-two type 2 diabetics with elevated post-prandial blood glucose (BG) above 200 mg/d (range: 253-350 mg/dL) were randomized to receive conventional diet, n=10 and automatic snacking, n=12. Total caloric requirement was fixed at 30kcal/kg/day divided into 3 meals and 2 optional snacks in the conventional diet and 3 meals and 3 snacks taken automatically 2 hours after each main meal even without hunger. Intensive insulin regimen given either as: combination of biphasic NPH 70/30 twice daily and aspart/lispro thrice daily premeals; or combination of glargine/detemir daily and aspart/lispro thrice daily initiated at 0.6units/kg/day and increased by 10-20% per day to meet target BG of 140-18-mg/dL. BG levels were measured using point-of-care glucose meter 2 hours after each main meal. Each patient was provided with a list of symptoms of hypoglycemia. They were instructed to take 150 mL of juice or soda upon onset of symptoms of hypoglycemia. Hypoglycemia monitoring was done.
Results: Patients on automatic snacking had fewer episodes of hypoglycemia compared to conventional diet therapy 8.3% vs. 60%, respectively. Likewise, there was lesser magnitude of hypoglycemia among those on automatic snacking than those on the conventional diet, (68 mg/dL vs 79 mg/dL). Patients in both groups were able to reach target BG of 140-180 mg/dL. Interestingly, patients who had automatic snacking reached target BG 1.2 days earlier than those on conventional diet p=0.005. In addition, those in the automatic snacking group had a greater reduction in the capillary blood glucose level from baseline BG than those in the conventional diet group with 47% and 34% reduction respectively p=0.04.
Conclusion: Automatic snacking prevents hypoglycemia among admitted type 2 diabetic patients on intensive insulin therapy.

Nothing to Disclose: ZGL, NERL, LBM-A

Pub # P2-528

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diabetes & Glucose Homeostasis: Genetic & Translational Approaches (1:30 PM-3:30 PM)

Title The Cardiovascular Biomarker GDF-15 Increases after LPS-Induced Endotoxemia in Healthy Men and Is Up-Regulated in PBMCs of Obese Patients

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Body Abstract Embargoed.

Nothing to Disclose: GV, MHR, MR, MC, AL

Pub # P2-529

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diabetes & Glucose Homeostasis: Genetic & Translational Approaches (1:30 PM-3:30 PM)

Title Associations between Serum 25-Hydroxyvitamin D and Indices of Hepatic and Whole-Body Insulin Sensitivity: Effects of Fasting Glucose Status

Author String AP Ashraf, JA Alvarez, WM Granger, BA Gower
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Body
Background: Serum 25-hydroxyvitamin D [25(OH)D] is known to influence glucose metabolism. Few studies have examined its relationship with insulin and glucose responses to a mixed meal challenge. Furthermore, it is not known if fasting glucose status influences the relationships between 25(OH)D and glucose metabolism. We aimed to identify the associations of serum 25(OH)D with serum glucose and insulin responses to a mixed meal tolerance test (MMTT), as well as to indices of insulin resistance and to determine if fasting glucose status influences these relationships.
Subjects and Methods: This was a cross sectional study involving 51 adults, ages 18- 55 years. The main outcome measures were fasting insulin, homeostasis model assessment of insulin resistance (HOMA-IR) and post-challenge insulin and glucose outcomes measured by a meal insulin sensitivity index (meal S_I) determined with mathematical modeling. Fasting insulin and HOMA-IR reflects hepatic insulin sensitivity. Meal S_I reflects both hepatic and skeletal muscle insulin sensitivity. Normal fasting glucose (NFG) was defined as <100 mg/dl and impaired fasting glucose (IFG) as 100-125 mg/dl.
Results: Mean 25(OH)D was 21.8 ± 9.8 ng/ml. Serum 25(OH)D was not significantly associated with meal S_I ($P > 0.15$) following a meal challenge. Serum 25(OH)D was associated with fasting insulin (standardized $\beta = -0.43$, $P = 0.002$) and HOMA-IR (standardized $\beta = -0.42$, $P = 0.003$), independent of age and sex; however the relationships were attenuated after further adjustment for percent body fat ($P = 0.10$ and 0.099 , respectively). Serum 25(OH)D was associated with HOMA-IR (standardized $\beta = -0.30$, $P = 0.06$), and fasting glucose (standardized $\beta = -0.46$, $P = 0.03$) independent of age, sex, and percent fat among participants with normal fasting glucose, but not in those with IFG.
Conclusions: 25(OH)D was associated with indices of hepatic insulin sensitivity, although the relationships may be mediated by adiposity. 25(OH)D was not associated with dynamic insulin sensitivity as reflected by meal S_I. Finally, 25(OH)D was associated with fasting glucose and HOMA-IR in subjects with NFG, but not in those with IFG, suggesting vitamin D may have preventative role in insulin resistance.

Nothing to Disclose: APA, JAA, WMG, BAG

Pub # P2-530

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diabetes & Glucose Homeostasis: Genetic & Translational Approaches (1:30 PM-3:30 PM)

Title The Artificial Sweetener Aspartame in Combination with Carbohydrate Reduces Insulin Levels during Endurance Exercise

Author String KG Howell, JC Siegler, NJ Thatcher, JW Bray, CP Towlson, DJ Peart, DD Mellor, R Vince, SL Atkin
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Body Aim. To determine the effect of aspartame ingestion with carbohydrate on blood glucose and insulin secretion during endurance exercise.
Methods: Nine healthy male volunteers each completed four separate trials of 60 min intense, self-paced cycling. Forty-five minutes prior to exercise, the subjects ingested 7ml[middot]kg⁻¹ body weight of: 1) water alone (W); 2) 0.04% aspartame with 2% carbohydrate (AC1); 3) 5% carbohydrate (C); 4) 0.04% aspartame with 5% carbohydrate (AC2). At 15 min intervals during exercise, participants ingested 2 ml[middot]kg⁻¹ body weight of their prescribed drinks. The average drink intake was 1491ml (1365-1685ml) giving an average aspartame intake of 596mg (546-675mg), aspartame intake equating to that contained in approximately 1L of a soft drink. Blood glucose (BG) was monitored every 10 minutes during exercise. Serum insulin was obtained pre-exercise, 30 min into exercise and immediately post-exercise. Subjects undertook each trial at one week intervals.
Results: Insulin levels dropped significantly for the AC2 versus C alone by 43% (p<0.01, CA 18.3 ± 5.1, 7.8 ± 2.0*; C 16.4 ± 2.8, 13.6 ± 3.7* pre-exercise and 30min respectively), whilst for W and A1 insulin levels did not differ. There was a significant increase of BG during the 60 min of exercise (p < 0.001), with both AC2 and C conditions maintaining BG better than W and AC1 from 40 - 60 min (p < 0.05: blood glucose levels at 10 min versus 60 min respectively for C 3.4 ± 0.1 vs 4.4 ± 0.2; CA 3.1 ± 0.1 vs 4.3 ± 0.3; W 3.5 ± 0.3 vs 3.3 ± 0.2; A 3.9 ± 0.1 vs 3.8 ± 0.2).
Conclusions: The combination of aspartame with 5% carbohydrate led to significantly lower serum insulin levels during exercise than carbohydrate alone, though BG levels did not differ in these healthy volunteers. In subjects taking exogenous insulin this potentially could lead to exercise induced hypoglycaemia if a combination of aspartame and carbohydrate were taken, and the threshold for this effect and the mechanisms for this action needs to be determined.

Nothing to Disclose: KGH, JCS, NJT, JWB, CPT, DJP, DDM, RV, SLA

Pub # P2-531
Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diabetes & Glucose Homeostasis: Genetic & Translational Approaches (1:30 PM-3:30 PM)
Title Study of Impact of Iron Indices on Glycemic Status, HOMA IR, HOMA B, Total Antioxidation Capacity and Inflammatory Markers in Patients with Uncontrolled Type 2 Diabetes Mellitus in Comparison with Healthy Controls and Patients with Type 2 Diabetes Mellitu
Author String R Jayanthi, MI Shaik, S Jayanthi
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Body **Introduction:** Iron deficiency anemia and diabetes mellitus are widely prevalent noncommunicable diseases in India.

Understanding how iron deficiency anemia influences glucose metabolism and insulin kinetics will help in elucidating the effects of IDA on the pathophysiological abnormalities in diabetes.(4)

Methods and materials: A crosssectional study was carried out in 30 healthy controls, 30 Diabetes mellitus and 30 Diabetes mellitus with iron deficiency anemia(IDA).Patients were BMI, Age, Sex matched newly detected diabetics.**Glycemic indices**, fasting serum insulin levels,HOMA- IR, HOMA-B (1-3),**Iron indices,Inflammatory markers,Total Antioxidation capacity(TAOC),Mitochondrial oxidative capacity (MOC)** were estimated.

RESULTS: In uncontrolled type 2 diabetes Mellitus in comparison with controls there was significant (p<0.001) increase in free iron,transferrin saturation, transferrin, FBS,Fasting Insulin,HbA1C HOMAIR,TNF alpha(p<0.001). Significant (p<0.001)decrease was found in HOMA B, TAOC,MOC In **diabetes mellitus with Iron deficiency anemia** in comparison with uncontrolled type 2 Diabetes Mellitus there was **significant (p<0.001) decrease** in iron, transferrin saturation, ferritin,FBS,HbA1C, insulin resistance ,TNF alpha (p<0.09) and increase in Interlukin- 6(p<0.05).

Discussion :

In **Uncontrolled Diabetes mellitus without IDA** Increased free iron levels (4,5,6),TNF-alpha contributed to increased oxidative stress, HOMA IR and decreased HOMA B .The low mitochondrial oxidative capacity shows that iron overload impairs mitochondrial function leading to impaired insulin secretion.

Decrease in insulin resistance in Diabetes mellitus with iron deficiency anemia compared to uncontrolled diabetes mellitus is due to decrease in serum iron, ferritin and increase in IL-6 and decrease in TNFalpha contributed to decrease in HOMA IR.

Conclusion:

This suggest that injudicious self supplementation with Iron by patients may have an adverse impact or the glycemic control.

The improved Insulin resistance and glycemic status in patients with DM and IDA may give falsely gratifying impression about the status of Diabetes control in such patients.

Further studies are needed to see whether these changes are reversible especially, after iron supplementation.

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Nothing to Disclose: RJ, MIS, SJ

Pub # P2-532

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diabetes & Glucose Homeostasis: Genetic & Translational Approaches (1:30 PM-3:30 PM)

Title Effect of Carvedilol and Metoprolol on Endothelial Function, Vascular Compliance, and Exhaled Nitric Oxide (FENO) in Patients with Type 2 Diabetes Melitus (T2DM) and Stage 1 Hypertension

Author String G Mahajan, K Soe, R Rana, N Winer
State University of New York Health Science Center at Brooklyn, Brooklyn, NY

Body Carvedilol is an α -1/ β -1, β -2 adrenergic receptor blocker with antioxidative properties, compared to metoprolol, a selective β -1 adrenoceptor blocker. The α / β antagonist may exert beneficial hemodynamic effects either by increasing endothelial nitric oxide (NO)-production or decreasing NO-breakdown. Twenty-eight patients with T2DM and hypertension, receiving angiotensin-converting enzyme inhibitors or angiotensin receptor blockers, were randomized on an intention-to-treat basis to receive either carvedilol (Coreg CR) (n=14) or metoprolol (Toprol XL) (n=14) **in graded doses over 12 weeks to achieve a goal blood pressure (BP) [le]130/80 mmHg. Groups did not differ in mean age (57 \pm 2), BMI (32.5 \pm 1.2 vs. 32.6 \pm 1.6), HbA1c (7.5 \pm 0.2 vs. 7.7 \pm 0.4), serum lipids, and duration of T2DM or hypertension. Mean BP fell from 142 \pm 1/85 \pm 2 to 132 \pm 3/79 \pm 2 mmHg on Coreg and from 142 \pm 3/83 \pm 3 to 136 \pm 5/76 \pm 3 on Toprol. The reactive hyperemia index (RHI) (Endopat 2000), a measure of endothelial function, was relatively unchanged by either Coreg or Toprol treatment. Large artery elasticity (Cardiovascular Profiling Instrument) increased from 9.4 \pm 0.7 to 12.6 \pm 1.4 mL/mmHg x 10 on Coreg and from 9.9 \pm 1.2 to 14.8 on Toprol, whereas small artery elasticity increased slightly with Coreg and declined slightly with Toprol. Systemic vascular resistance decreased significantly from 1723 \pm 53 to 1664 \pm 65 (p<.001) and from 1740 \pm 69 to 1708 \pm 107 dynes /sec/cm⁵ on Toprol. Coreg and Toprol increased FENO from 20.4 \pm 2 to 24.4 \pm 2 and from 19.1 \pm 2.5 to 28 \pm 3.6 ppb (p<.01), respectively. We conclude that both Coreg CR and Toprol XL lower BP effectively, but lack a significant effect on endothelial function. Both drugs improve large artery elasticity, while Toprol has an adverse effect on small vessel elasticity. Coreg significantly lowers systemic vascular resistance. Both agents increase exhaled NO, suggesting a possible increase in systemic NO release or reduced NO bioinactivation.**

Nothing to Disclose: GM, KS, RR, NW

Pub # P2-533

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diabetes & Glucose Homeostasis: Genetic & Translational Approaches (1:30 PM-3:30 PM)

Title Osteoblast in Hyperglycemia: Alteration of Differentiation

Author String MG Lara-Cardoso, G Barbosa-Sabanero, LM Salgado-Rodriguez, M Sabanero-Lopez
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Body Diabetes mellitus is a chronic degenerative disease that has reached alarming levels in our country [1]. It is characterized by several complications [2], among which is the damage to the bone. Several reports suggest that diabetes mellitus can be one of the causes of osteopenia and osteoporosis [3]. Bone damage is mainly due to alterations in bone homeostasis, as reflected in the damage to the functionality of its constituent cells, particularly the osteoblast. This cell type is actively involved in bone formation through differentiation. During this cellular event different specific markers are expressed [4]. However, are unknown effects of hyperglycemia in osteoblasts at cellular level.

Objective. To test osteoblast differentiation process under high concentrations of glucose in the medium with presence and absence of insulin.

Methods. Human osteoblast cultures (ATCC MG-63) were grown in osteogenic medium (ascorbic acid 50 microg/ml, b-glycerophosphate 7.5 mM) [5], low glucose and fetal bovine serum 10% and incubated at 37 [deg] C [6]. Osteoblasts were exposed to different concentrations of glucose (5, 15, 25 mM) and treated or not with insulin (0.6 nM) for 48 and 72 h [5]. Quantification of alkaline phosphatase activity and the formation of calcium nodules were analyzed, just as the calcium concentration in the nodules formed by osteoblasts exposed to the different experimental conditions mentioned above.

Results. The results show that alkaline phosphatase activity decreases as a function of glucose concentration. In particular, the stage of mineralization is impaired, as reflected by a low amount of calcium nodules formed and a lower concentration of calcium in them. Furthermore, insulin treatment shows an increase in the expression of these markers, but do not reach the levels shown by osteoblasts exposed to physiological concentrations of glucose.

Conclusions. These data suggest that osteoblast differentiation process is impaired by hyperglycemia. However, it is necessary to evaluate the mechanisms by which these changes occur, to achieve a new understanding of the biology of osteoporosis in diabetic patients.

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Nothing to Disclose: MGL-C, GB-S, LMS-R, MS-L

Pub # P2-534

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diabetes & Glucose Homeostasis: Genetic & Translational Approaches (1:30 PM-3:30 PM)

Title High Prevalence of Hypogonadotropic Hypogonadism (HH) in Type 2 Diabetes

Author String R Nemr, A Al-Katheer, A Echtay, WY Almawi
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Body Previous studies showed that type 2 diabetes (T2DM) is associated with a high prevalence of hypogonadotropic hypogonadism (HH), and cross-sectional studies identified low testosterone and increased LH and FSH concentrations to be frequently linked with type 2 diabetes. Whereas HH is associated with features of the metabolic syndrome (obesity, hypertension and hyperlipidemia) in T2DM patients, it was not related to T2DM duration or HbA1c levels. The aim of this study was to determine the prevalence of HH in T2DM patients in a primary healthcare facility. We investigated the extent of HH in T2DM by measuring total FSH, LH, prolactin (PRL) and testosterone (T) concentrations in 163 T2DM patients, and correlated changes in sex hormone levels with obesity, age, HbA1c levels, and T2DM and duration. Hypogonadism was defined as low testosterone. The mean age was 58.3 ± 12.0 years, mean BMI was 29.8 ± 4.8 kg/m², and mean HbA1c was $8.6 \pm 1.4\%$. Mean T was 10.41 ± 1.9 nmol/L, FSH was 23.20 ± 4.8 nmol/L, and LH was 10.6 ± 2.8 nmol/L. Of the 163 patients, 61 (37.4%) were hypogonadal. LH (40.5 ± 9.1 vs. 29.9 ± 6.3 IU/L; $P < 0.001$) and FSH (15.6 ± 2.9 vs. 7.6 ± 1.6 IU/L; $P < 0.001$) levels were significantly higher in the hypogonadal compared with eugonadal patients. Testosterone was negatively correlated with BMI ($r = -0.371$; $P < 0.01$), but not with age or disease duration. LH ($r = -0.421$; $P < 0.001$) and FSH ($r = -0.498$; $P < 0.001$) levels correlated negatively with T. The decline of testosterone concentrations was calculated at 2.42 nmol/L per decade. In conclusion, our results indicate high prevalence of subnormal free testosterone concentrations among T2DM patients, which is more pronounced on obese vs. non-obese cases. This supports earlier studies demonstrating frequent occurrence of HH in T2DM.

Nothing to Disclose: RN, AA-K, AE, WYA

Pub # P2-535

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diabetes & Glucose Homeostasis: Genetic & Translational Approaches (1:30 PM-3:30 PM)

Title Experimental Sleep Restriction Reduces Physical Activity in Adults with Parental History of Type 2 Diabetes

Author String L Bromley, JN Booth III, L Alcantar, J Imperial, PD Penev
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Body Adults with parental history of type 2 diabetes have a high risk of developing the disease, particularly in the setting of physical inactivity and excessive weight gain. Self-reported sleep duration < 6 h/day has been associated with increased incidence of diabetes, and sleep-loss-related reduction in physical activity has been hypothesized to contribute to the association of short sleep with metabolic morbidity. Our study tested the hypothesis that experimental sleep restriction will result in reduced daily physical activity in individuals at increased risk of developing type 2 diabetes.

Eleven healthy adults with parental history of type 2 diabetes (7F/4M, mean [SD] age 26 [2.5] y, BMI 24.0 [2.5] kg/m²) completed two 7-day inpatient studies with scheduled bedtimes of 8.5 vs. 5.5 h/night in random crossover fashion 4 to 12 weeks apart. Participants who exercised regularly at home (36%) were allowed to maintain their usual exercise patterns during each study period. Daily sleep was measured by wrist actigraphy (Actiwatch-64, Mini Mitter Co.) and total body movement was measured continuously by waist accelerometry (Actical, Mini Mitter Co.) during both study periods. Mixed models analysis was used to compare the total number of daily activity counts and the time spent in light, moderate and vigorous-intensity physical activity between the two sleep conditions.

Sleep time was reduced by 142 [22] min/day during the 5.5-h time-in-bed condition (288 min/day [20] vs. 430 min/day [41]; P<0.01). Total daily activity counts declined by 32% (P<0.05) during the period of experimental sleep restriction. This was accompanied by a 19% reduction in moderate-intensity physical activity (P<0.01) and a 22% reduction in combined moderate plus vigorous-intensity physical activity (P<0.02). There was no significant difference in light intensity physical activity between the two sleep conditions.

In conclusion, experimental sleep restriction in healthy adults with parental history of type 2 diabetes resulted in reduced total daily body movement and time spent in moderate plus vigorous-intensity physical activity. Our results support the hypothesis that sleep-loss-related reduction in physical activity can contribute to the risk of developing type 2 diabetes in susceptible individuals.

Sources of Research Support: NIH grants R01-HL089637, CTSA-RR 024999 and P60-DK020595.

Nothing to Disclose: LB, JNB, LA, JI, PDP

Pub # P2-536

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diabetes & Glucose Homeostasis: Genetic & Translational Approaches (1:30 PM-3:30 PM)

Title D-Lactate and Its Metabolic Intermediates as Potential Biomarkers for Diabetic Vascular Complications

Author String KJ Forke, B Ling, G Zello, TG Arnason
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Body Diabetes mellitus (DM) is a common metabolic disease, the complications of which are of significant concern. Specifically, microvascular (neuropathy, retinopathy, renal failure) and macrovascular (heart disease, stroke) diseases can be fatal or debilitating. The mechanism of these complications remains undefined, however vascular lesion progression has been associated with endothelial dysfunction. We are interested in determining if the natural products of high blood glucose metabolism directly impact on development of diabetic complications, specifically in Type 2 DM (DM2). Baseline levels of normal glucose metabolites in patients with and without DM2 will be quantified. Methylglyoxal (MG), a metabolite of glucose, is typically significantly elevated in diabetic patients, and has been considered important in the development of vascular complications. MG is metabolized to D-lactate (DLA) through what is considered to be a detoxification pathway. It has been established that diabetic patients have a higher basal level of DLA, and DLA levels can be further raised by Metformin (MET) treatment. It has recently been reported that DLA can negatively affect the energy profile of brain and heart cells. We hypothesize that long-term exposure to subclinical levels of D-lactate (levels not causing acidosis) contribute to vascular dysfunction in diabetic patients, and may be a significant contributor to vascular complications. The impact of MET and DM2 on baseline DLA and other glucose metabolites will be assessed by recruiting patients from 4 groups: DM2 treated with MET, DM2 without MET, and non diabetics taking MET for other purposes. A control group (no DM2, no MET) will also be included. Ethics and study design have been approved by both Saskatoon Health Region and Royal University Hospital/U of S. Biochemical analysis will be carried out on blood leftover from routine testing. Routine measurements through the hospital laboratory will include, glucose, insulin, HgA1c, and cholesterol profile. Our lab analysis will measure DLA, L-lactate, MG and pyruvate levels using enzymatic kits from Biovision. Understanding the relationships between these compounds in diabetic and non diabetic patients may help to begin uncovering the mechanism behind diabetic disease progression and vascular complications. These metabolites may potentially serve as an important biomarker for diabetic complications in the future. Results of study to be presented at conference.

Nothing to Disclose: KJF, BL, GZ, TGA

Pub # P2-537

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diabetes & Glucose Homeostasis: Genetic & Translational Approaches (1:30 PM-3:30 PM)

Title Regulation of Lipogenesis in Human Hepatocytes by Cortisol and Insulin

Author String M Nasiri, LL Gathercole, D Hauton, PM Stewart, JW Tomlinsons
University of Birmingham, Birmingham, UK

Body Glucocorticoid (GC) excess (Cushing's syndrome) is characterized by central obesity, insulin resistance and in up to 20% of cases, non-alcoholic fatty liver disease (NAFLD). NAFLD is a progressive spectrum of disease ranging from hepatic steatosis to steatohepatitis, fibrosis and cirrhosis. Many processes contribute to lipid accumulation within hepatocytes including de novo lipogenesis which includes the rate-limiting carboxylation of acetyl CoA to malonyl-CoA by acetyl CoA carboxylase (ACC) and conversion to palmitate by fatty acid synthase (FAS). We have hypothesized that increased GC exposure drives insulin resistance and promotes the development of NAFLD.

C3A cells (human hepatoma derived), which express the GC receptor, were treated with cortisol at varying concentrations (100-1000nM) in the presence or absence of insulin (80nM, 24h). Lipogenic gene expression was determined by real-time PCR and lipogenesis measured by 1-[14C] acetate incorporation into triglyceride.

Cortisol increased mRNA expression of ACC2 (0.005±0.003 (control) vs. 0.008±0.004 (cortisol), p<0.05) and FAS (0.071±0.007 (control) vs. 0.110±0.019 (cortisol), p<0.05) but not ACC1, diacylglycerol acyl transferase (DGAT) and glycerol phosphate acyl transferase (GPAT). However, cortisol decreased functional lipogenesis in a dose dependent manner (85.66.6% [100nM], 73.57.9% [250nM], 55.045.6% [1000nM] vs. control (100%), p<0.01) probably reflecting rate-limiting, post-transcriptional regulation. Interestingly, in the absence of cortisol, insulin only had a modest effect to increase lipogenesis and this did not reach statistical significance (114.9±12.4% vs. control (100%), p=ns). In contrast, when C3A cells were pre-treated with cortisol for 24h, the stimulatory effect of insulin upon lipogenesis was augmented (mean % increase in lipogenesis following insulin, 14.9±12.4% (control) vs. 38.2±12.3±% (cortisol) [250nM], p<0.05).

In C3A cells, in the absence of insulin, GCs decreased lipogenesis despite increasing FAS and ACC2 expression. Consequent increased hepatic free fatty acid exposure may contribute to hepatic insulin resistance. However, our data also suggest that GCs and insulin may act synergistically to promote lipid storage in hepatocytes.

Nothing to Disclose: MN, LLG, DH, PMS, JWT

Pub # P2-538

Session Information POSTER SESSION: CLINICAL - Diabetes: Cardiometabolic Risk (1:30 PM-3:30 PM)

Title Adolescents with Type 2 Diabetes (T2D) Show Early Cardiovascular Impairment with Exercise

Author String TE Pinto, S Gusso, JGB Derraik, JC Baldi, WS Cutfield, PL Hofman
Liggins Institute, University of Auckland, Auckland, New Zealand; Northern Arizona University, Flagstaff, AZ

Body Maximal exercise capacity may be limited as a result of T2D, irrespective of deconditioning in both adults and adolescents. This may be secondary to central (cardiac) and/or peripheral (vascular) abnormalities. **Aims:** 1) determine exercise capacity (VO_{2peak}) in a group of adolescents with T2D compared to an obese, non-T2D group and a non-obese control group; 2) to assess cardiac function in these groups with the use of cardiac magnetic resonance imaging (MRI) at rest and during sub-maximal exercise; 3) to assess femoral artery flow (FAF) at rest and immediately post-exercise as a marker of peripheral vascular function. **Methods:** 13 subjects with T2D, 27 overweight or obese subjects and 19 non-diabetic, non-obese controls, aged 12-20 year were recruited. All subjects performed an incremental exercise test on a cycle ergometer. Body composition was determined using dual-energy x-ray absorptiometry (DEXA). Cardiac and femoral flow MRI images were taken at rest and during or immediately after sub-maximal exercise using a cycle ergometer designed for use in the MRI. **Results:** Maximum heart rate (HR) achieved was higher in the control group than in the two other groups ($p < 0.01$) despite comparable maximum workloads. There was no difference in cardiac output (CO) indexed for fat free mass (FFM) between groups at rest however, during exercise CO/FFM was lower in the T2D compared to the obese and control groups ($p < 0.01$, $p < 0.001$ respectively). SV increased by 11.1% in the control group, 5.98% in the obese group and 0.76% in the T2D group during exercise. End-diastolic volume (EDV)/FFM was significantly lower in the T2D both at rest and during exercise. During exercise, EDV decreased by 2.1% in the control group, 0.9% in the obese group and 6.1% in the T2D group. End-systolic volume (ESV)/FFM was lower in the T2D group at rest and during exercise compared to controls ($p < 0.01$). The average FAF/minute and the net forward volume both indexed for FFM were significantly lower in the T2D group post exercise compared to the other 2 groups ($p < 0.05$). **Conclusion:** Independent of obesity, T2D negatively affects both central and peripheral vascular function during exercise in adolescents. Central causes appear to be secondary to impaired filling and possible diastolic dysfunction, while peripheral blood flow to exercising muscles may also be impaired. Both may hinder one's ability to engage in physical activity.

Sources of Research Support: Novo Nordisk (unrestricted grant); Canadian Pediatric Endocrine Group Scholarship (Hoffmann-La Roche).

Nothing to Disclose: TEP, SG, JGBD, JCB, WSC, PLH

Pub # P2-539

Session Information POSTER SESSION: CLINICAL - Diabetes: Cardiometabolic Risk (1:30 PM-3:30 PM)

Title Predictive Value of Specific Measures of Body Composition in the Assessment of Insulin/Glucose Homeostasis after Dextrose Ingestion in Healthy Men

Author String D Lawson, J Veldhuis, B Dunn, A Iranmanesh
Salem VA Medical Center, Salem, VA; Mayo Clinic, Rochester, MN

Body Body mass index (BMI), percentage body and abdominal fat are interchangeably used as temporal correlates of insulin release and sensitivity. The present study was intended to assess the predictive value of BMI, fat mass index (FMI), and visceral fat area (VFA) on insulin sensitivity and glucose/insulin concentration profile: in 47 healthy men in the age range of 19-78 yrs, BMI 20-39 Kg/m², FMI 1.8-11.2 Kg/m², and VFA 14.4-410 cm². Total body fat mass (FM) was computed by DXA. Fat mass index (FMI) was derived by dividing FM in Kg by height in meter-squared. CT scan was used to estimate visceral fat area (VFA). Each subject was studied on 2 separate occasions after an overnight fast, consisting of either 75 grams of oral dextrose solution or equal volume of water. Sessions started between the hours of 0800-0900 and continued for a total period of 6.5 hrs, with blood collected at 10-min intervals for the measurements of glucose and insulin concentrations. HOMA-IR was calculated using serum glucose and insulin concentrations in a single fasting blood specimen, and ranged from 0.4--5.5. Data are presented as mean ±SE. Sum of 6.5-hour glucose and insulin concentrations after dextrose ingestion increased from 3495±43 to 3733±79 mg/dL (P=0.0002), and 183±18 to 739±60 [micro]U/mL (P<0.0001), respectively. Backward stepwise multiple regression statistics with BMI, FMI, and VFA as independent variables identified FMI as a preferred predictor (R²/P) of HOMA-IR (0.43/<0.0001), as well as the sum (0.34/<0.0001), mean (0.34/<0.0001), peak (0.25/<0.0001), and nadir (0.19/0.0006) glucose concentrations. FMI was similarly more predictive of changes in sum (0.27/0.0003), and mean (0.27/0.0003) insulin concentrations, as well as time to glucose nadir (0.25/<0.0001) and time to recovery of glucose to baseline values (0.08/0.03). Whereas BMI was universally inferior, VFA was preferred in estimating time to peak glucose (0.11/0.012), and time to peak insulin (0.09/0.05) concentrations. In summary, FMI appears to be more predictive of the metabolic effect of body composition on glucose and insulin homeostasis, including insulin sensitivity. Compared with BMI, FMI has the advantage of offsetting the heterogeneity introduced by age, muscle mass and fat distribution. While for the most part being superior to VFA, FMI is also more readily available, is less costly, and has a significantly smaller risk of radiation exposure.

Nothing to Disclose: DL, JV, BD, AI

Pub # P2-540

Session Information POSTER SESSION: CLINICAL - Diabetes: Cardiometabolic Risk (1:30 PM-3:30 PM)

Title Effect of Diabetes and Exercise on NF[κ]B-Regulated Genes in Skeletal Muscle

Author String M Li, P Tantiwong, A Sriwijitkamol, DK Coletta, N Musi
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Body Nuclear factor [κ] B (NF[κ]B) is a transcription factor that controls the expression of numerous proinflammatory proteins. Type 2 diabetes (T2DM) is characterized by skeletal muscle insulin resistance and muscle atrophy, and NF κ B has been implicated in the pathogenesis of these disorders. Exercise is important for the treatment and prevention of diabetes and muscle atrophy, however, the molecular mechanism by which exercise leads to these beneficial effects is still unclear. In this study we examined whether insulin resistant subjects have abnormal gene expression of NF κ B subunits and NF κ B-regulated genes in skeletal muscle. We also determined the effect of aerobic exercise on these genes. A vastus lateralis muscle biopsy was performed in 11 lean (BMI=24.8 \pm 0.6 kg/m²; age=40 \pm 3 y, FPG=93 \pm 2 mg/dl, VO₂max=21 \pm 1 ml/kg.min), 6 obese (BMI=30.4 \pm 1; age=42 \pm 4, FPG=94 \pm 2, VO₂max=18 \pm 1), and 9 obese T2DM (BMI=34.1 \pm 0.6; age=51 \pm 4, FPG=133 \pm 14, VO₂max=15 \pm 1) subjects before and after a short-term aerobic training program. mRNA expression of IKK, NF[κ]B p50, NF κ B p65, NF κ B p100, TLR4, MCP-1 and SOD2 was determined by real-time RT-PCR. The training program consisted of 14 consecutive days of cycle exercise for 40 min/day at 70% to 90% VO₂max. Results: Training increased VO₂max by 19%, 17%, and 14% in lean, obese, and T2D subjects, respectively. Baseline gene expression of IKK beta (230%), NF κ B p100 (140%), NF κ B p65 (40%), and TLR4 (70%), MCP-1 (140%) was significantly increased in T2DM subjects (P<0.05 vs. lean). The basal gene expression of several of these inflammatory proteins (IKK beta, NF κ B p100, NF κ B p65, TLR4 and MCP-1) also tended (P=NS) tended to be increased in obese individuals. Exercise significantly increased mRNA levels of IKK beta (30%) and NF κ B p100 (50%) in lean subjects. Exercise also increased TLR4 mRNA in lean (140%) and obese (80%) subjects. MCP-1 expression increased in lean (430%), obese (220%), and T2DM (140%) subjects, whereas SOD2 expression increased mainly in the obese group (60%). In summary: (1) T2DM subjects have increased gene expression of several proteins within the TLR4-IKK-NF[κ]B axis; and (2) short-term exercise stimulates TLR4-NF[κ]B signaling, although this effect is blunted in T2DM subjects, possibly because baseline expression of these genes is already elevated in these individuals.

Sources of Research Support: NIH grant AG030979; NIH grant DK080157; the American Federation for Aging Research.

Nothing to Disclose: ML, PT, AS, DKC, NM

Pub # P2-541

Session Information POSTER SESSION: CLINICAL - Diabetes: Cardiometabolic Risk (1:30 PM-3:30 PM)

Title Prompt Increases in Serum RBP4 and Endothelial Progenitor Cells Following Exercise Load in Type 2 Diabetic Subjects

Author String A Aoki, M Murata, T Asano, M Sasaki, A Ikoma, M Kawakami, S-e Ishikawa
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Body Retinol-binding protein 4 (RBP4) is the primary carrier for retinol in plasma, and synthesized in liver and adipose tissues. Endothelial progenitor cells (EPCs) derived from bone marrow are involved in regulating vascular endothelial cells. The present study was undertaken to determine whether acute exercise load change serum RBP4 and EPCs in type 2 diabetic subjects. 62 type 2 diabetic subjects (65.1±8.1 years) were enrolled. Acute exercise loaded until maximal oxygen intake was performed, and serum RBP4 and EPCs were determined before and after the exercise load. Serum RBP4 was measured by ELISA, and CD34+/133+ cells as EPCs were determined by FACS. The subjects were subgrouped by diabetic nephropathy. Serum RBP4 levels, but not the numbers of EPCs, were increased according to the progression of diabetic nephropathy (stage1: 48.2±23.5, stage2: 47.4±12.9, stage3: 54.1±23.2, stage4: 87.3±13.5 p value in trend=0.043) In the 30 subjects without nephropathy (stage 1), an exercise load promptly increased serum RBP4 from 48.2±23.5 to 54.3±23.1 [μ g/ml (p=0.0006). In the 32 subjects with nephropathy (stage 2, 3, and 4) the exercise load did not alter serum RBP4. By contrary, an exercise load significantly increased the numbers of EPCs in both the diabetic subjects with stage 1 (88.9±83.3 to 114.9±104.4 cells/100[μ l], p=0.0003), and stage 2, 3 and 4 (63.0±68.4 to 78.5±68.8 cells/100[μ l], p=0.005). The alteration in serum RBP4 ([Δ]RBP4) during the exercise had a positive correlation with eGFR (r=0.30 p=0.018). Also, the numbers of EPCs and the alteration of EPCs ([Δ]EPCs) during the exercise had positive correlations with serum RBP4 levels (the numbers of EPCs: r=0.402, p=0.006; [Δ]EPCs: r=0.310, p=0.04). These findings indicate that an increase in serum RBP4 in response to acute exercise load is only kept in type 2 diabetic subjects without nephropathy, though serum RBP4 increases according to the progression of diabetic nephropathy. RBP4 may participate in maintenance of vascular endothelial function in association with induction of EPCs.

Nothing to Disclose: AA, MM, TA, MS, AI, MK, S-EI

Pub # P2-542

Session Information POSTER SESSION: CLINICAL - Diabetes: Cardiometabolic Risk (1:30 PM-3:30 PM)

Title Basic Fibroblast Growth Factor Predicts Cardiovascular Disease Occurrence in a Subset of Participants in the Veterans Affairs Diabetes Trial

Author String M Zimering, RJ Anderson, L Ge, TE Moritz, C Abaira, W Duckworth, Investigators for the VADT Veterans Affairs New Jersey Healthcare System, East Orange, NJ; Veterans Affairs Hospital, Hines, IL; Miami Veterans Affairs Medical Center, Miami, FL; Phoenix Veterans Affairs Healthcare System, Phoenix, AZ

Body Intensive diabetes treatment slows the progression of retinopathy, nephropathy and neuropathy, however, its role in reducing macrovascular events in adult type 2 diabetes is less clear. The aim of the Veterans Affairs Diabetes Trial (VADT) was to test whether intensive glucose- lowering reduces cardiovascular disease occurrence in adults with differing durations of diabetes. The current VADT substudy examined whether plasma basic fibroblast growth factor predicts cardiovascular disease (CVD) occurrence in nearly four-hundred men, age 40 years or older, mean : hemoglobin A_{1c} 9.5%, diabetes duration 11.4 years, BMI 31 kg/m², and 37% of whom reported a baseline macrovascular event. Subjects were randomly assigned to intensive or standard glycemic treatment lasting up to seven and one-half years. Plasma basic fibroblast growth factor (bFGF) was determined at baseline study entry (n=399) and after one-year of treatment in 215 randomly-selected subjects. Cardiovascular disease (CVD) occurrences were adjudicated by an independent endpoints committee. One hundred-five first cardiovascular events occurred in 399 subjects. The best fit model of risk factors associated with the time to first cardiovascular disease occurrence during up to 7.5 years of follow-up had as significant predictors: duration-treatment interaction (p =.03), baseline plasma basic fibroblast growth factor (p =.01), age (p =.03), and prior CV event (p < .0001). Intensive glucose-lowering was associated with significantly decreased hazard ratios for CVD occurrence (0.38-0.63) in patients with baseline diabetes durations ranging from 0-10 years; and hazard ratios not significantly different from 1 (0.82-1.78) in patients with diabetes durations ranging from 15-30 years. Increased year 1 bFGF ($\geq 4.5\text{pg/mL}$) was nearly significantly associated with time to first post-year 1 CVD occurrence (HR 2.439, p = 0.05) in a high risk subset of patients with (≥ 15 years) baseline diabetes duration. These data suggest that plasma bFGF predicts CVD occurrence in a subset of VADT participants. Intensive glucose- lowering may reduce cardiovascular disease events in patients with ten or fewer years' type 2 diabetes duration. Factors related to increased post-treatment plasma basic fibroblast growth, e.g. renin-angiotensin system activation, may contribute to the ongoing risk for CVD occurrence in subsets of long-standing type 2 diabetes.

Sources of Research Support: Veterans Biomedical Research Institute, East Orange, NJ; Cooperative Studies Program of the Department of Veterans Affairs, Office of Research and Development, Washington, DC.

Nothing to Disclose: MZ, RJA, LG, TEM, CA, WD

Pub # P2-543

Session Information POSTER SESSION: CLINICAL - Diabetes: Cardiometabolic Risk (1:30 PM-3:30 PM)

Title Nontraditional Risk Factors and Vascular Disease Progression in Diabetic and Nondiabetic Recipients after Kidney Transplantation

Author String J Larsen, C Borgeson, E Lyden, F Yu
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Body Introduction. Cardiovascular disease (CVD) is the greatest cause of mortality after kidney transplantation (KTX). Nontraditional risk factors have become the focus of CVD risk of KTX patients. Methods. In an ongoing prospective, observational clinical trial we have evaluated the role of nontraditional risk factors in CVD progression after KTX, as indicated by carotid intima media thickness (CIMT), with an emphasis on the role of inflammation and vitamin D/PTH. Eligibility included [ge]6 mo post-KTX and GFR >30 ml/min. A number of traditional and nontraditional risk factors are being evaluated. Results. In this cohort (n=342; 57% M/43%F), 24% had type 1 diabetes (DM1), 11% type 2 (DM2), 15% post-transplant DM (PTDM), and 50% no diabetes (nonDM). Mean age was 52.6±0.6y (± SEM) and time since KTX was 5.92±0.33 y (range 0.5-33.8y). 309 completed 1 y, 269 completed 2 y, and 176 have completed 3 y to date. Baseline CIMT was 0.622±0.006 mm (range 0.422-1.081) and significantly increased in the cohort over time (p<0.0001). Of those completing 3 years follow-up, mean change in carotid IMT was 0.055±0.006mm (range: -0.213- +0.357). When we compared baseline criteria between those with greater (>0.04mm) and less 2 y CIMT progression (<0.04mm), only PTH (p=0.03) and hsCRP (p=0.059) were significant or nearly significant between these 2 groups among many CVD risk factors (e.g.,age, gender, ethnicity, BMI, BP, diabetes status, smoking status, GFR, A1C, vitamin D, lipids, urine alb/Cr, HOMA-IR, time since KTX, and homocysteine). Using a mixed-effects regression model, smoking status (p=0.0027), hsCRP (p=0.01), LDL (p=0.018), and total cholesterol (p=0.04) were significant univariate predictors of CIMT progression over the three years and pulse pressure (p=0.07) and diabetes status (p=0.09) were borderline significant. hsCRP at baseline is associated with BMI, triglycerides, and diabetes status (p<0.05). Conclusions: Inflammation is associated with CVD progression after KTX, in addition to traditional risk factors, in an ongoing observational trial. While the analysis is ongoing, among the causes of inflammation are BMI and features of insulin resistance.

Nothing to Disclose: JL, CB, EL, FY

Pub # P2-544

Session Information POSTER SESSION: CLINICAL - Diabetes: Cardiometabolic Risk (1:30 PM-3:30 PM)

Title Racial/Ethnic Differences in the Relationship between Uric Acid and Fasting Insulin in Adolescent Females: NHANES 1999-2006

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Body Uric acid is strongly associated with insulin resistance and the metabolic syndrome among adolescents and high levels of uric acid are an independent predictor of long-term risk for cardiovascular disease and Type 2 Diabetes Mellitus (T2DM). While it is known that insulin resistance and T2DM are more prevalent in non-Hispanic-black and Hispanic individuals than in non-Hispanic whites, it is not known whether the association between uric acid and insulin resistance varies by gender and race/ethnicity. We used NHANES data ('99-'06) and performed linear regression to evaluate associations between uric acid and fasting insulin (a marker of insulin resistance) in adolescents on a gender- and race/ethnicity basis. We evaluated 12-19 y.o. adolescents who were non-Hispanic white, non-Hispanic black or Hispanic, adjusting for age, education and household income. We then generated model-based estimates of uric acid as a continuous predictor of fasting insulin levels, evaluating fasting insulin levels at uric acid levels of 3.5, 5 and 6.5 mg/dL (approximately the 10th, 50th and 90th percentile of the cohort). Using our regression model among adolescent females, a uric acid level of 3.5 mg/dL was associated with similar fasting insulin levels among the three racial/ethnic groups (all groups <9 IU/mL). However, a uric acid 5 mg/dL was associated with higher insulin among non-Hispanic-black (12.56[CI 11.2-14.1]) than non-Hispanic-white (9.25[8.27-10.34]) but not Hispanic females (11.0[9.7-12.5])(p<0.05). Moreover, a uric acid of 6.5 was associated with higher insulin among both non-Hispanic-black (17.7[14.7-21.5]) and Hispanic (14.5 [11.4-18.4]) females compared to non-Hispanic whites (10.14[8.2-12.5])(p<0.05). Using this model among males revealed no significant differences in fasting insulin levels by race/ethnicity at any level of uric acid. We conclude that high levels of uric acid are more strongly associated with high levels of insulin in non-Hispanic-black and Hispanic females than non-Hispanic white females, while there were no racial/ethnic differences observed in the relationship uric acid and insulin in males. Given the importance of insulin resistance in risk for future T2DM, these findings may bear significance for racial/ethnic differences among females in the long-term association of uric acid with future disease.

Sources of Research Support: 5K08HD060739-02 (MDD) and 1R21DK085363 (MJG and MDD).

Nothing to Disclose: MDD, MJG

Pub # P2-545

Session Information POSTER SESSION: CLINICAL - Diabetes: Cardiometabolic Risk (1:30 PM-3:30 PM)

Title Decreased Insulin Sensitivity, but Not Beta-Cell Dysfunction, Is the Primary Cause of Rising Fasting Blood Glucose in Glucose-Tolerant Subjects

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Body Impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) are two intermediate metabolic stages between normal glucose tolerance (NGT) and diabetes (DM). Evidence suggested impaired basal insulin secretion as fundamental pathophysiology of IFG and reduced secondary insulin response in IGT. Little is known about the transitional stage from NGT to IFG. We examined the relationship between beta cell function (BCF) and insulin sensitivity (IS) among subjects with high normal fasting blood glucose concentration (FBG).
We analyzed the data from participants age 18 years old or older in the National Health and Nutrition Examination Survey (NHANES) 2007-2008, a cross sectional survey of the U.S. population. Only the subject with glucose tolerance and insulin results are included in this analysis. We excluded the subjects who are taking insulin or anti-diabetic medications. The sample consists of 352 male and 448 female. We quantified IS (HOMA-S) and BCF (HOMA-B) by using the homeostatic model assessment from fasting glucose and insulin concentrations. Subjects were divided into three groups according to their FBG; less than 90 mg/dl (n=217, group A), 90-94 mg/dl (n=257, group B), and 95-99 mg/dl (n=326, group C).
HOMA-S decreased as FBS increased with significant differences among three groups ($P<0.001$). In order to compare changes in BCF, raw data was logarithmic transformed into linear regression, as BCF reflected dynamic changed according to IS. The slopes were fairly similar among three groups (-0.9858 for group A, -0.9850 for group B, -0.9847 for group C). These results indicated that in spite of worsening IS, beta cells compensated in the same fashion among three groups. As the slopes are almost identical among three groups, the significant differences in intercept merely reflected the difference in HOMA-S ($P<0.00001$).
In the present study, we observed a significant decline in IS as FBG increased with a trend of decrease, but no different statistically, in the compensation of BCF. We concluded worsening IS was the primary cause of the raising FBG in glucose tolerant subjects.

Nothing to Disclose: RK, H-YO, L-MC, KCC

Pub # P2-546

Session Information POSTER SESSION: CLINICAL - Diabetes: Cardiometabolic Risk (1:30 PM-3:30 PM)

Title Treatment of Severe Lipodystrophy with Metreleptin in a Patient with Active Juvenile Dermatomyositis

Author String SL Amarnath, S Kamineni, A Neidert, Y Kalagara, B Adams, Y Kalagara, JL Chan, EA Oral
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Body Juvenile dermatomyositis (JDM) is a rare autoimmune disease. Ten to 40% of patients with JDM have acquired lipodystrophy (LD). While administration of recombinant human leptin (metreleptin) has been shown to correct metabolic abnormalities in LD due to other causes, efficacy in this form of LD has not been studied, particularly as leptin appears to exacerbate autoimmunity in rodents. We are now reporting the effect of metreleptin therapy for >15 months in a child with active JDM and generalized LD within the context of an expanded access program of metreleptin for LD patients with diabetes mellitus and/or hypertriglyceridemia sponsored by Amylin Pharmaceuticals (San Diego, CA). Patient presented at age 6 with joint pains, fatigue, weight loss and increased appetite. Physical exam showed coarse physical features, decreased facial and body fat, hepatomegaly, increased muscle bulk, negative Gower's sign, and increased skin pigmentation. Labs showed CPK of 477 U/L, aldolase 16.7 U/L, triglycerides 14,370 mg/dl, HDL 31 mg/dl, HbA1C 9.7%, insulin 975 uIU/mL and leptin 0.67 ng/mL. MRI of the lower extremities showed increased T2 signal in her large muscles demonstrating active inflammation. Diagnoses of JDM and generalized LD were made. Her diabetes was treated with metformin, pioglitazone, acarbose and insulin (up to 320 units daily) but remained poorly controlled (HbA1c>9%). Despite aggressive therapy, her triglycerides remained >10,000 mg/dL, and she was hospitalized with recurrent acute pancreatitis. At age 9, she was started on metreleptin at a dose of 0.04 mg/kg/day (0.7 mg), which was titrated to 0.2 mg/kg/day (4mg) over the next 12 months. Her HbA1c decreased from 9.2% to 7.2%, her insulin requirement decreased from 320 units to 40 units daily and her triglyceride levels decreased to 114 mg/dL at 15 months of therapy. She maintained either stable or improved muscle enzyme levels and enhanced functional capacity as well as overall muscle strength. She developed vaginal spotting at age 10 years and 3 months (bone age 13 years, height 165 cm) while on metreleptin therapy, which became regular over 3 months. This is the first case of LD with active JDM treated with metreleptin therapy. Metreleptin was associated with substantial improvements in glycemic control and hypertriglyceridemia despite reductions in diabetes therapy without any apparent adverse effects on her autoimmune disease or functional capacity.

Disclosures: EAO: Employee, Amylin Pharmaceuticals. Nothing to Disclose: SLA, SK, AN, YK, BA, YK, JLC

Pub # P2-547

Session Information POSTER SESSION: CLINICAL - Diabetes: Cardiometabolic Risk (1:30 PM-3:30 PM)

Title Circulating Maternal Androgens during and after Pregnancy: Relationship with Insulin Resistance

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Body An association between circulating androgen levels and insulin resistance has been demonstrated in women with the polycystic ovary syndrome. In pregnant women, this relationship has not been clearly investigated. Objective: To examine the association between maternal androgen levels and glucose homeostasis during and after pregnancy while also considering the confounding effect of body mass index (BMI). Methods: The study included 47 pregnant women (20 women with gestational diabetes (GDM) and 27 controls) tested using a 75g oral glucose tolerance test. Glucose and insulin responses were quantified using areas under the curves of glycemia and insulinemia over 120 minutes after the glucose load. Insulin sensitivity was also estimated by calculating the Matsuda and HOMA indices. Plasma concentrations of testosterone, dihydrotestosterone (DHT) and dehydroepiandrosterone (DHEA) were measured by high performance gas chromatography and chemical ionization mass spectrometry at the time of GDM screening (26.1±3.7 wks) and 2 months after pregnancy (8.7±0.8 wks). Results: In every woman, testosterone and DHT levels were significantly higher during pregnancy than after delivery (3.03±1.87 vs. 0.55±0.30 nmol/L and 0.48±0.24 vs. 0.18±0.11 nmol/L respectively, p[le]0.001). There was a trend for an association between pre-pregnancy BMI and plasma testosterone levels measured during pregnancy (r=0.24, p=0.10). No significant difference was observed for testosterone, DHT or DHEA between women with or without GDM, either during or after pregnancy. However, glycemic response, Matsuda and HOMA indices were significantly associated with testosterone levels during pregnancy (r=0.30, r=0.35 and r=-0.37 respectively, p[le]0.05 for all). The association with the Matsuda index remained significant after adjustment for pre-pregnancy BMI. Glycemic response, the Matsuda and HOMA indices were also significantly associated with DHEA levels during pregnancy (r=0.29, r=0.29 and r=-0.29 respectively, p[le]0.05 for all), which remained significant after adjustment for pre-pregnancy BMI. After pregnancy, only testosterone levels were significantly associated with the glycemic response and Matsuda index (r=0.30 and r=-0.30, p[le]0.05). This association was no longer significant after adjustment for BMI measured post-partum. Conclusion: These results suggest that elevated testosterone and DHEA levels are observed in conjunction with insulin resistance during pregnancy.

Nothing to Disclose: A-SM, M-CD, FL, JR, JSW, AT

Pub # P2-548

Session Information POSTER SESSION: CLINICAL - Diabetes: Cardiometabolic Risk (1:30 PM-3:30 PM)

Title Effects of First-Year Growth on Body Composition (BC), Resting Energy Expenditure (REE) and Metabolic Profile Differ in Preterm (PT) Children Born with Very Low Birth Weight (VLBW) Either Appropriate (AGA) or Small for Gestational Age (SGA)

Author String C Sepulveda, E Pittaluga, C Urquidi, A Avila, G Iniguez, F Carrasco, V Mericq
Faculty of Medicine, University of Chile, Santiago, Chile; Hospital Dr Sótero del Río, Santiago, Chile;
Faculty of Medicine, University of Chile, Santiago, Chile; Faculty of Medicine, University of Chile, Santiago Chile

Body Fast growth in first 3 months of life adversely affects metabolic risks of young adults born at term. Controversy exists with regard to differences in metabolic risk in PT born SGA or AGA. Goal: To determine whether there are differences in BC, REE and metabolic variables between children born VLBW either AGA or SGA and whether these differences are related to a certain period of weight gain during the first year of life. 67 VLBWPT (<32 weeks / <1500g), (40 AGA, 27 SGA). Followed as part of the National Program for VLBWPT infants from age 40 weeks until 7 yrs. BC by DEXA (Lunar DPX-L), REE by indirect calorimetry using the method Canopy (Deltatrac [reg]) and blood sampling at age 6.7±0.5 yr. Continuous variables: mean and SD and t Student test (independent groups). Categorical variables, frequencies and proportions compared by X² (for independent groups). To assess the relationship of the different growth periods and body composition and energy expenditure: linear regression models with body composition and REE as dependent variables and the changes of weight SDS and length SDS as independent variables, were built. All these data were analyzed by a multivariate analysis. VLBW SGA children were leaner (p<0.05), shorter (p<0.01) and had lower waist and hip circumferences (p<0.005), HDL Chol (p<0.05) and higher % fat (p<0.05), % region of interest fat (ROI) (p<0.04), trunk fat (p<0.01) than their AGA counterpart (adjusted by age, sex, BMI). Weight gain patterns differed between SGA and AGA VLBW (p<0.001). The difference is observed by CA 0 and 3 months of life. After adjusting for age, gender and adequacy at birth (SGA/AGA) there was a direct correlation between weight gain in the first 3 months and total fat, % total fat, % ROI, % trunk fat and inversely with REE and FFM. Weight gain between 6-9 months in SGA was correlated with total and % fat mass and ROI whereas in AGA correlates only with REE and REE/fat free mass. Weight gain between 9-12 months in SGA was correlated with total and % fat mass and trunk fat. By age 6 yr lower BW+higher fat mass subjects had higher Insulin and leptin (p<0.001). Summary: There were significant differences between SGA/AGA VLBW children in anthropometry, BC and calorimetry and these differences were correlated to early periods of growth. All periods of weight gain in SGA are correlated to fat mass whereas in AGA 6-9 months of weight gain are correlated with REE. We speculate that the difference in this period may be due to higher lean mass in AGA children.

Sources of Research Support: Pfizer Individual Grant.

Nothing to Disclose: CS, EP, CU, AA, GI, FC, VM

Pub # P2-549

Session Information POSTER SESSION: CLINICAL - Diabetes: Cardiometabolic Risk (1:30 PM-3:30 PM)

Title Anthropometric Variables Accurately Predict DXA-Derived Body Composition and Can Be Used To Screen for Diabetes and Metabolic Syndrome

Author String R Yavari, E McEntee, M Brines
Yale School of Medicine and Beyond Care, New Haven, CT; George Washington University School of Medicine, Washington, DC; Beyond Care, Guilford, CT

Body

Background: We previously examined DXA body scans looking for predictors of weight loss (Endo 2007.) In this study we analyzed scans of female subjects enrolled in a lifestyle change program for predictors of diabetes type 2 (DM) and metabolic syndrome (MS). It is known that anthropometrics can be used for DM, MS and cardiovascular risk assessment. Here, an anthropometric-based model (ANTHRO) was developed to predict body composition. Next, the DXA-derived and ANTHRO models were used separately to predict DM and MS in a large female population.

Method: Total fat mass (FM), trunk fat (TF) and fat free mass (FFM) of 341 female subjects were studied by DXA. Subjects were randomly split into a cross-validation group (CV; n=100) and an index group (IG; n=241). Using anthropomorphic measurements as independent variables, a stepwise regression model (ANTHRO) was developed to predict FM, TF, and FFM by DXA. The ANTHRO model was generated in the IG and validated in the CV group. Next, a logistic regression model was constructed using DXA data as independent variables and the presence or absence of DM and MS as dependent variables. Finally, the ANTHRO model was used to screen for the DM and MS in a population of 1153 female patients of an endocrinology practice.

Results: The ANTHRO model estimated DXA-determined FM, TF and FFM with accuracy. The difference between ANTHRO-predicted vs. DXA-measured FM had a mean of zero and a standard deviation (SD) of 3.1 kg. Similarly, TF and FFM had means of zero and SDs of 2.1 and 3.1 kg. Hipline but not waistline correlated to FM. Both correlated to TF, FFM and weight. When other variables were held constant, waistline correlated with FFM suggesting females with central obesity were muscular.

The DXA-derived logistic model showed good discrimination for DM based on a receiver-operating characteristic with an area under the curve (AUC) of 0.78. The ANTHRO model was similar with an AUC of 0.81. Both models yielded better AUCs than screening questionnaires and were comparable to the AUC of a model based on HgA1c.

Conclusions: A DXA-derived predictive model has good discrimination to detect DM and MS in a population of females with a wide range of BMI. An anthropometric-based model of body composition also can similarly assess DM and MS risks. Both non-invasive models predict risk comparably to HgA1c but the anthropometric model is faster and less costly. Further refinement of the model is underway in other populations.

Nothing to Disclose: RY, EM, MB

Pub # P2-550

Session Information POSTER SESSION: CLINICAL - Diabetes: Cardiometabolic Risk (1:30 PM-3:30 PM)

Title The Risk of Overall Mortality in Patients with Type 2 Diabetes Receiving Different Combinations of Sulfonylureas and Metformin: A Retrospective Analysis

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Cleveland Clinic, Cleveland, OH

Body
Background: Some studies have reported an increased risk of mortality with sulfonylurea and metformin combination therapy; however, most analyzed the sulfonylureas as a medication class (1-3). The individual sulfonylureas vary significantly from one another in terms of hypoglycemic risk, sulfonylurea receptor selectivity, and their effects on myocardial ischemic preconditioning (4,5). These differences in properties may be important when using sulfonylureas in conjunction with metformin, which inhibits gluconeogenesis (6). A few studies have suggested that glyburide, which inhibits ischemic preconditioning and has the highest risk of hypoglycemia among sulfonylureas, confers a greater mortality risk than other sulfonylurea and metformin combinations (7,8).
Purpose: To assess the risk of overall mortality in patients with type 2 diabetes treated with different combinations of sulfonylureas and metformin.
Methods: A retrospective cohort study was conducted using an academic health center enterprise-wide electronic health record (EHR) system to identify 7,320 patients with type 2 diabetes (3,768 initiators of glyburide and metformin, 2,277 initiators of glipizide and metformin, and 1,275 initiators of glimepiride and metformin), seen in the outpatient clinics, [ge] 18 years of age, and not on insulin or a non-insulin injectable a baseline. The patients were followed for mortality by documentation in the EHR and Social Security Death Index. Multivariable Cox models with propensity scores were used to compare cohorts.
Results: Baseline characteristics for the entire cohort include mean age +/- SD of 62.1 +/- 12.4 years, 53.3% male, and 78.4% Caucasian. The median follow-up was 2.4 years. There were a total of 636 deaths in 18,870 person years of follow-up. No statistically significant difference in the risk of overall mortality was observed among the different combinations of sulfonylureas and metformin: glimepiride and metformin vs. glipizide and metformin (HR 1.14; 95% CI 0.89-1.44, P=0.30), glimepiride and metformin vs. glyburide and metformin (HR 1.22; 95% CI 0.97-1.53, P=0.08), or with glipizide and metformin vs. glyburide and metformin (HR 1.08 95% CI 0.90-1.28, P=0.41).
Conclusions: Our results did not identify an increased mortality risk among the different combinations of sulfonylureas and metformin, suggesting overall mortality is not substantially influenced by the choice of sulfonylurea.

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Disclosures: RSZ: Speaker, Novo Nordisk, Bristol-Myers Squibb, Astra Zeneca. Nothing to Disclose: KMP, MWK, CY, BJW, SA, BN, AJ, AA

Pub # P2-551

Session Information POSTER SESSION: CLINICAL - Diabetes: Cardiometabolic Risk (1:30 PM-3:30 PM)

Title PNPLA3 Polymorphism Influences Liver Fibrosis in Unselected Patients with Type 2 Diabetes

Author String JM Petit, B Guiu, D Masson, L Duvallard, MC Brindisi, B Bouillet, P Buffier, JP Cercueil, P Hillon, B Verges
CHU du Bocage, Dijon, France; INSERM, Dijon, France

Body **Context-** Recently, it has been shown that an allele in the adiponutrin (PNPLA3) gene was strongly associated with increased liver fat content, and liver fibrosis independently of visceral adiposity and insulin resistance. **Objective -** In this study, we set out to determine whether PNPLA3 rs738409 polymorphism was associated with liver fibrosis in unselected patients with type 2 diabetes. **Design, Setting, and Participants -** 234 type 2 diabetic patients were included in this study. **Main Outcome Measures-** Liver fat content was evaluated using ¹H-MR Spectroscopy, fibrosis was measured using the non-invasive FibroTest[reg]. **Results -**an advanced liver fibrosis (stage superior or equal to F2) was observed in 10.2 % patients while 149 (63.6 %) patients had steatosis. Prevalence of steatosis and fibrosis was higher in minor G allele carriers than in C allele homozygote carriers (70.3% vs 57.1%; p=0.04 and 14.7% vs. 7.5%; p=0.07, respectively). In multivariate analysis, the predictive variables for advanced liver fibrosis were age(>60) [P = 0.005], sex (male) [P=0.004], and rs 738409 PNPLA3 polymorphism [P=0.01]; BMI, and liver fat content were not associated with liver fibrosis **Conclusions -** this study confirms that in patients with type 2 diabetes who were not selected for liver abnormalities, liver fibrosis was related to rs738409 polymorphism independently of BMI or liver fat content.

Nothing to Disclose: JMP, BG, DM, LD, MCB, BB, PB, JPC, PH, BV

Pub # P2-552

Session Information POSTER SESSION: CLINICAL - Diabetes: Cardiometabolic Risk (1:30 PM-3:30 PM)

Title Serum Selenoprotein P Levels in Patients with Impaired Glucose Tolerance and Type 2 Diabetes: Implication for Insulin Resistance, Inflammation and Atherosclerosis

Author String SJ Yang, HC Hong, HY Choi, MJ Cho, YJ Kim, JH Kim, CR Eun, HJ Yoo, HY Kim, JA Seo, NH Kim, SG Kim, SH Baik, DS Choi, KR Choi
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Body **OBJECTIVE :** The dysregulation of hepatokine may be closely associated with the pathogenesis of insulin resistance and type 2 diabetes. Recent studies suggested a role of selenoprotein P (SeP), a novel hepatokine, in the regulation of glucose metabolism and insulin sensitivity. We examined the relationship between circulating SeP levels and clinical parameters associated with insulin resistance in humans.
RESEARCH DESIGN AND METHODS : We compared serum SeP concentrations in subjects with normal glucose tolerance (NGT, n = 30), impaired glucose tolerance (IGT, n = 45), and type 2 diabetes (n = 45). Furthermore, we evaluated the relationship between SeP and cardiometabolic risk factors including insulin resistance, high sensitivity C-reactive protein (hsCRP), and carotid intima-media thickness (CIMT).
RESULTS: Serum SeP concentrations showed a stepwise increasing trend according to glucose tolerance status ($P = 0.017$) and were higher in patients with type 2 diabetes compared with those with IGT or NGT (median 1104.9 [573.3-2711.5], 774.9 [452.2-1519.7], and 442.5 [252.5-1161.1], respectively). Subjects with overweight and obesity demonstrated increased SeP levels compared to lean subjects ($P = 0.010$). Age- and gender-adjusted correlation analysis showed significant relationship between SeP and cardiometabolic factors including waist circumference, blood pressure, triglycerides, glucose, liver function test, and insulin resistance. Furthermore, in multiple regression analyses, SeP exhibited independent association with CIMT as well as hsCRP even after adjustment of other risk factors.
CONCLUSIONS: Circulating SeP concentrations were found to be elevated according to glucose metabolism dysregulation and to be related to various cardiometabolic parameters including insulin resistance inflammation, and atherosclerosis.

Nothing to Disclose: SJY, HCH, HYC, MJC, YJK, JHK, CRE, HJY, HYK, JAS, NHK, SGK, SHB, DSC, KMC

Pub # P2-553

Session Information POSTER SESSION: CLINICAL - Diabetes: Cardiometabolic Risk (1:30 PM-3:30 PM)

Title The Relationship between Heart-Type Fatty Acid Binding Protein and Carotid Artery Intima-Media Thicknes in the Early Diagnosis of Cardiac Injury and Atherosclerosis in Patients with Impaired Glucose Tolerance and Impaired Fasting Glucose

Author String B Karbek, M Ozbek, Z Ginis, N Colak Bozkurt, A Gungunes, I Ozturk Unsal, E Cakal, T Delibasi Di&Skapi Yildirim Beyazit Teaching and Research Hospital, Ankara, Turkey; Di&Skapi Yildirim Beyazit Teaching and Research Hospital, Ankara, Turkey

Body Heart type fatty acid binding protein (H-FABP) is a major cytoplasmic low-molecular weight protein and released into the circulation when the myocardium is injured (1). Previous studies have demonstrated that H-FABP is closely associated with acute coronary syndrome, hypertrophic and dilated cardiomyopathy, heart failure, stroke, obstructive sleep apnea syndrome, pulmonary embolism. The aim of this study was to investigate serum H-FABP volue and to evaluate the difference in carotid artery intima-media thickness(IMT) between patients with impaired glucose tolerance (IGT) or impaired fasting glucose (IFG), and control subjects. In the present study, we tested the hypothesis that serum H-FABP level is detectable in the circulation of patients with prediabetes, and may be associated with subclinical atherosclerosis. Study was performed at the department of Endocrinology in Diskapi Yildirim Beyazit Trainig and Research Hospital, during the period January 2010- july 2010. 58 subjects with prediabetes-29 with IGT, of mean age 46,9±10,4 years and 29 with IFG, of mean age 45,2±10,3 years- and a group of 28, age , gender and BMI matched subjects with normal glucose tolerance (NGT) were enrolled. Categories of glucose tolerance were defined applying 2006 WHO criteria (2). LDL, HDL cholesterol, triglycerides and fasting serum insulin were estimated, Weight and height of the subjects were measured and body mass index (BMI) and homeostatic model assessment (HOMA-IR) were calculated. We measured serum H-FABP levels in 58 prediabetic patient: (29 with IFG, 29 with IGT) and 28 age-sex-BMI matched control subjects by using a sandwich enzyme-linker immunosorbent assay. High-resolution B-mode ultrasonography was conducted in all participants. Serum H-FABP levels were significantly elevated in prediabetic patients when compared with control subjects, respectively, (IFG- 32,5±34,2 ng/dl IGT- 45,4±45,8 ng/dl control- 16,8±14,9 ng/dl p:0.011). The patients with IGT and IFG groups had higher levels of H-FABP compared to the control group. (p=0,010, p=0,009). CIMT were significantly higher in prediabetic groups than control group (IFG- 0,6±0,1, IGT- 0,6±0,1, control-0,5±0,1 p<0,001). A significant positive correlation was found between H-FABP and CIMT (p<0,001, rho=0,626). Patients with prediabetes have an increased risk of cardiovascular diseases. Serum H-FABP levels might be a marker of myocardial performans in patient with IFG and IGT.

(1) N.C. Fournier, M.A. Richard, Role of fatty acid-binding protein in cardiac fatty acid oxidation. Mol. Cell. Biochem. 98, 149-159 (1990)

(2) The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 2000;23:S4-S19.

Nothing to Disclose: BK, MO, ZG, NCB, AG, IOU, EC, TD

Pub # P2-554

Session Information POSTER SESSION: CLINICAL - Diabetes: Cardiometabolic Risk (1:30 PM-3:30 PM)

Title Values of Endothelial Progenitor Cells in Predicting Coronary Artery Disease in Patients with Type 2 Diabetes Mellitus

Author String I Kocaoglu, S Isik, M Falay, S Aktas, O Ucar, U Ozuguz, S Gokaslan, D Berker, S Aydogdu, S Guler
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Body

Objective: The importance of balance between endothelial damage and repair in cardiovascular events is well known. Risk of cardiovascular disease has been increased in patients with diabetes mellitus (DM). There is a need for better quantitative indicators to determine patients with type 2 diabetes mellitus (T2DM) having higher cardiovascular risk. The objective of this study is to determine whether endothelial progenitor cells (EPC) may be a predictor for coronary artery disease (CAD) in patients with T2DM.

Methods: Seventy-nine patients with T2DM without known CAD and 20 healthy individuals as control group underwent coronary angiography (CAG). Blood samples were drawn at the time of CAG and EPCs were measured by flow cytometry.

Results: Mean ages of patients were significantly higher compared with the control group (59.6 vs. 30.3 years). Of the patient group, 68.4% were female and 31.6% were male, and of the control group, 50% were female and 50% were male. In the patient group 39.2% (n=31) were found to have normal coronary arteries and 60.8% (n=48) had CAD. The patient group had significantly lower EPC count than the controls (4.80 vs. 11.33 cells/ μ l; $p < 0.001$). The EPC count was lower in patients with hypertension (HT) (4.69 vs 8.06 cells/ μ l, $p < 0.001$) compared to nonhypertensive or smokers compared to nonsmokers (5.21 vs 8.46 cells/ μ l, $p = 0.027$). Negative correlation was observed between EPC count and age, DM duration, and fibrinogen level ($r = -0.321$, $p < 0.001$; $r = -0.334$, $p = 0.019$ and $r = -0.312$, $p = 0.018$, respectively). After corrections for HT, age, and smoking, EPC count had negative correlation only with the presence of DM ($\beta = -0.886$, $p < 0.001$). In the diabetic group EPC count was correlated only with duration of DM ($r = -0.334$, $p = 0.019$). EPC count was similar in DM patients with or without CAD ($p = 0.219$). There was no correlation between EPC and CAG score within the patient group ($r = -0.091$ and $p = 0.427$).

Conclusion: In our study we have shown that EPC count was lower in patients with T2DM compared with the control group and this difference persisted even after adjustments for other variables. A significant negative correlation was found between DM duration EPC. However, a difference was not observed between individuals diagnosed with or without CAD and EPC among patients with T2DM. Further studies are needed to determine whether EPC count may be a predictor for CAD in T2DM patients.

Nothing to Disclose: IK, SI, MF, SA, OU, UO, SG, DB, SA, SG

Pub # P2-555

Session Information POSTER SESSION: CLINICAL - Diabetes: Cardiometabolic Risk (1:30 PM-3:30 PM)

Title The Impact of Socioeconomic Status on Mortality Following Acute Cardiovascular Events among Persons with Diabetes

Author String PRY Bishara, AS Bierman, LL Lipscombe, BR Shah, D Feig, O Bhattacharya, GL Booth
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Body

Background: Low socioeconomic status (SES) is a predictor of cardiovascular disease (CVD) and all-cause mortality in the general population and a risk factor for poor outcomes in those with diabetes.

Methods: We undertook a population-based study in Ontario Canada, using administrative health data to examine the impact of income on cardiovascular outcomes in the diabetic population. The Ontario Diabetes Database (ODD), a validated registry created from hospitalization and ambulatory service claims, was used to identify adults [ge] age 20 with diagnosed diabetes who were living in Ontario on April 1, 2002 and were admitted to hospital for acute myocardial infarction (AMI) or stroke during a follow up period ending March 31, 2008 (N=30,280). Income quintiles were assigned to each using area-level data from the 2001 Canadian Census. Logistic regression was used to examine the impact of income on mortality from any cause at 30 days and 1 year following admission. Because out-of-pocket costs of medications and supplies may contribute to income-related differences in health outcomes in the outpatient setting, we stratified each analysis by age, since those [ge]65 years receive coverage under the province's government-funded drug benefit program while those <65 years do not.

Results: The mean age of the cohort at baseline was 61.8 years, and 51.9% were male. There was no significant income gradient in mortality 30 days after AMI. However 1 year mortality following AMI was significantly higher in the lowest income quintile (OR 1.12; 95%CI 1.04-1.20) compared with the highest. Adjustment for age, sex, baseline CVD and number of the health care visits did not affect this association. The magnitude of the income gradient in 1 year mortality following AMI was more pronounced in patients under age 65 (OR=1.33;95% CI 1.09-1.63) compared to patients aged 65+ (OR 1.08; 95% CI 1.00-1.18). There was no income gradient in mortality at 30 days or one year following stroke.

Conclusion: Our study found a strong inverse relationship between income and mortality 1 year following AMI in younger and middle-aged adults with diabetes. It reinforces the need for aggressive risk reduction strategies together with a focus on improving chronic disease management in low income groups with diabetes. Further study around the role of drug reimbursement in reducing income-related differences in CVD is needed.

Sources of Research Support: 1-Ontario Ministry of Health and Long-Term Care; 2-Improving Women's Health in Ontario (ECHO).

Nothing to Disclose: PRYB, ASB, LLL, BRS, DF, OB, GLB

Pub # P2-556

Session Information POSTER SESSION: CLINICAL - Diabetes: Cardiometabolic Risk (1:30 PM-3:30 PM)

Title Lipocalin-2 in Relation to Anthropometric Measurements and Metabolic Risk Factors, at Baseline and at Two-Year Follow-Up, in the Cyprus Metabolism Study

Author String X Liu, O-PR Hamnvik, M Petrou, H Gong, JP Chamberland, C Christophi, S Kales, DC Christiani, CS Mantzoros
Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA; Brigham and Women's Hospital, Harvard Medical School, Boston, MA; Cyprus International Institute for Environmental and Public Health, Limassol, Cyprus; Harvard School of Public Health, Boston, MA; Boston VA Healthcare System, Boston, MA

Body **Background:** It has been suggested that lipocalin-2 is a link between obesity and insulin resistance, but both animal and cross-sectional studies in humans exploring these associations have revealed conflicting results. The few cross-sectional human studies have limitations including the inclusion of only middle-aged subjects and no adjustment for renal function, which is known to affect lipocalin-2 levels. There have been no prospective studies to date.
Objective: We studied the relationships between serum levels of lipocalin-2 and metabolic parameters at baseline and prospectively at 2-year follow-up in young healthy men. Similarly, associations were explored for total and free leptin levels, used herein as positive controls.
Research subjects and methods: 272 participants were randomly selected from the Cyprus Metabolism Study (916 18-year-old men), including all 91 subjects who participated in the follow-up study 2 years after baseline assessment.
Results: In the cross-sectional study, we found significant positive correlation between levels of lipocalin-2 and measures of central fat distribution (waist circumference, $r=0.15$, $p=0.017$), and triglycerides ($r=0.18$, $p=0.004$), but not other anthropometric parameters. After correcting for age, fat mass, waist to hip ratio, smoking status, and exercise, levels of lipocalin-2 were found to be significantly inversely correlated with fasting insulin ($r=-0.19$, $p=0.002$), and homeostasis model of assessment-insulin resistance (HOMA-IR) ($r=-0.16$, $p=0.01$). After further adjusting for creatinine levels, lipocalin-2 remained significantly inversely correlated with fasting insulin ($r=-0.19$, $p=0.002$) only. In prospective analyses, baseline levels of lipocalin-2 were not a predictive of any variables 2 years of follow-up in the fully adjusted model. As expected, total and free leptin were associated with BMI, all measures of adiposity, blood pressure, fasting glucose and lipid measurements.
Conclusions: We demonstrated that lipocalin-2 is associated with central fat distribution and weakly and inversely with insulin, but not insulin resistance after adjusting for potential confounders including renal function. In addition, lipocalin-2 is not an independent predictor of metabolic and cardiovascular risk factors in young men prospectively.

Nothing to Disclose: XL, O-PRH, MP, HG, JPC, CC, SK, DCC, CSM

Pub # P2-557

Session Information POSTER SESSION: CLINICAL - Diabetes: Cardiometabolic Risk (1:30 PM-3:30 PM)

Title Diabetic Retinopathy Predicts All-Cause Mortality and Cardiovascular Events in Both Type 1 and 2 Diabetes Mellitus: A Meta-Analysis of Observational Studies

Author String CK Kramer, TC Rodrigues, LH Canani, JL Gross, MJ Azevedo
Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil

Body The association of diabetic retinopathy (DR) with death and/or cardiovascular events has been investigated, but its predictive role for these outcomes is still debatable. Therefore, the aim of this study was to investigate the association of DR with all-cause mortality and cardiovascular events in patients with type 2 and type 1 diabetes by a systematic review and meta-analysis. The electronic databases Medline and Embase were searched up until April/2010 for cohort studies that evaluated DR in type 2 or type 1 diabetic patients and reported total mortality and/or fatal and non-fatal cardiovascular events (myocardial infarction, angina pectoris, myocardial revascularization procedures, ischemic changes on a conventional 12-lead electrocardiogram, transient ischemic attack, nonfatal stroke, or lower leg amputation). Two independent reviewers performed data extraction. Pooled effect estimates were obtained by using random-effect meta-analysis. A total of 20 studies fulfilled inclusion criteria and were included, providing data from 19,234 patients. In patients with type 2 diabetes (n = 14,896) the presence of any degree of DR increased the risk for all-cause mortality and/or cardiovascular events by 2.34 (95%CI 1.96-2.80) as compared with patients without DR. The positive likelihood ratio of any DR for all-cause mortality and/or cardiovascular events was 1.78 (95%CI 1.57-2.00). In patients with type 1 diabetes (n = 4,438) the corresponding risk for all-cause mortality and/or cardiovascular events was 4.10 (95%CI 1.50-11.18) and the positive likelihood ratio was 1.80 (95%CI 1.20-2.70). In 15 included studies, the risk determined by DR was also adjusted for possible confounders. The results did not change when an additional meta-analysis including these adjusted odds ratios was performed. DR was also predictive for all-cause mortality in both type 2 (OR 2.41; 95%CI 1.87-3.10) and type 1 (OR 3.65; 95%CI 1.05-12.66) diabetes. Links between DR and studied outcomes were not addressed by the current meta-analysis, but it demonstrated that the presence of DR can help to identify patients with increased risk for adverse outcomes. In conclusion, DR predicts all-cause mortality and cardiovascular events in both type 2 and type 1 diabetes.

Sources of Research Support: Projeto de N[uc]cleos de Excel[en]cia do Ministério de Ci[en]cia e Tecnologia, Conselho Nacional de Desenvolvimento Científico e Tecnológico; Fundo de Incentivo a Pesquisa de Hospital de Clínicas de Porto Alegre (FIPE-HCPA); C.K.K is a recipient of a grant from Projeto Nacional de Pós-Doutorado no País (PNPD 03021/09-2).

Nothing to Disclose: CKK, TCR, LHC, JLG, MJA

Pub # P2-558

Session Information POSTER SESSION: CLINICAL - Diabetes: Cardiometabolic Risk (1:30 PM-3:30 PM)

Title Postprandial Studies Identify Early Precursors of Atherosclerosis in Subjects with Type 1 Diabetes

Author String ANM McGowan, WM Widdowson, A O'Connor, HM Roche, G Boran, K Moore, J Gibney
The Adelaide and Meath Hospital, Incorporating the National Children's Hospital, Tallaght, Ireland; The Adelaide and Meath Hospital, Incorporating the National Children's Hospital, Tallaght, Ireland; UCD Conway Institute of Biomedical Research, School of Public Health and Population Science, University College Dublin, Belfield, Ireland

Body Subjects with type 1 diabetes (T1DM) have an increased absolute and relative risk of cardiovascular morbidity and mortality. Endothelial dysfunction precedes the development of cardiovascular disease in diabetic and non-diabetic subjects, possibly mediated through inflammation. Postprandial glucometabolic changes impair endothelial function in normal and type 2 diabetic subjects but little data exists in subjects with type 1 diabetes (T1DM). Subjects with type 1 diabetes (n=11) and age, sex and BMI-matched controls (n=8) were studied under fasting conditions, and for 8 hours during which time they received 2 mixed-meals. Biochemical variables and flow-mediated dilatation (FMD), a non-invasive measure of endothelial function, were measured. Intestinally derived apolipoproteins (apo) B48 levels were measured using a novel ELISA. Fasting and postprandially, glucose concentrations were greater in diabetic subjects ($p < 0.001$ at all time points). There were no significant differences in triglyceride concentrations or apoB48 levels between groups. Under fasting conditions; white cell count, neutrophil count and FMD did not differ between groups. Postprandially, the mean neutrophil count increased significantly in diabetic subjects compared to controls ($3.52 \times 10^9/L$ to $4.69 \times 10^9/L$ versus $3.06 \times 10^9/L$ to $3.24 \times 10^9/L$, $p < 0.05$) and also compared to fasting values ($p < 0.05$). FMD decreased significantly in diabetic subjects only (6.66% to 2.48% versus 5.07% to 4.87%, $p < 0.001$ compared to controls and $p < 0.001$ vs. fasting) A significant correlation existed between postprandial neutrophil count, glucose AUC and % change in FMD postprandially ($r = 0.631$, $p < 0.01$ postprandial neutrophils and glucose AUC, $r = 0.577$, $p < 0.05$ postprandial neutrophils and % change in FMD and $r = 0.634$, $p = 0.005$ Glucose AUC and % change FMD). T1DM is associated with a specific inflammatory response and endothelial dysfunction postprandially which is possibly glucose mediated. These findings could contribute to the increased prevalence of atherosclerosis observed in this group. Further studies are needed to elucidate the pathophysiology of these effects.

Nothing to Disclose: ANMM, WMW, AO, HMR, GB, KM, JG

Pub # P2-559

Session Information POSTER SESSION: CLINICAL - Diabetes: Cardiometabolic Risk (1:30 PM-3:30 PM)

Title Sarcopenia Is Associated with Insulin Resistance and Early Phase of Type 2 Diabetes: The Korean Health and Genome Study (KHGS)

Author String JW Son, HK Jeong, SS Lee, SR Kim, SJ Yoo, NH Cho
The Catholic University of Korea, Bucheon, Korea; Ajou University School of Medicine, Suwon, Korea

Body
Objective - Sarcopenia can contribute to insulin resistance and an increased risk of type 2 diabetes. The aim of this study was to determine appropriate criteria for age-related sarcopenia and sarcopenic obesity (SO), reflected by the degree of insulin resistance, and to evaluate their associations with an increased risk of type 2 diabetes.
Methods - Our analyses included 1285 men and 1724 women who had complete data available for body composition analysis and the 75 g oral glucose tolerance test. Sarcopenia was assessed by the appendicular skeletal muscle mass (ASM)/height², the ASM/weight % or the skeletal muscle mass index (SMI). Obesity was identified based on the total body fat percentage or the body mass index (BMI).
Results - ASM/weight % and SMI, but not ASM/height², were inversely associated with the homeostasis model assessment of insulin resistance (HOMA-IR). The prevalence of sarcopenia and SO, defined as ASM/weight % less than the one S.D. below the sex-specific normal mean of a younger reference group and a BMI of over 25 kg/m², tended to be higher with increased HOMA-IR tertile. Compared to either sarcopenia or obesity alone, SO was associated with a higher risk of pre-diabetes and type 2 diabetes in those 60 years or older after adjusting for confounding factors. Subjects with sarcopenia were at especially high risk of newly diagnosed diabetes in older age groups.
Conclusions - Sarcopenia and SO, assessed by the ASM/weight % and BMI, were strongly associated with the degree of insulin resistance and the early phase of type 2 diabetes. These findings suggest that sarcopenia could be an important role in the progression from pre-diabetes to type 2 diabetes.

Nothing to Disclose: JWS, HKJ, SSL, SRK, SY, NHC

Pub # P2-560
Session Information POSTER SESSION: CLINICAL - Diabetes: Cardiometabolic Risk (1:30 PM-3:30 PM)
Title The Difference in Arterial Stiffness among Patients with Normoglycemia, Impaired Glucose Tolerance and Diabetes in Patients with Established Congestive Heart Failure
Author String PE Otterbeck, J Lazar, H Kamran, E Trofimovsky, L Saliccioli, J Kassotis, MA Banerji
SUNY Downstate Medical Center, Brooklyn, NY

Body **Introduction:** Diabetes is common in the setting of congestive heart failure and confers an adverse prognosis (1). Diabetes is associated with increased arterial stiffness, which in turn is predictive of higher CV event rates (2, 3). Whether undiagnosed diabetes and glucose intolerance are associated with higher arterial stiffness in the setting of CHF is unknown. The aim of this study is to determine the differences in arterial stiffness in patients with CHF across the spectrum of glycemic control at an urban city hospital.

Methods:

Patients and procedures: Using outpatient clinic registries, 71 African-American patients with stage 1-3 heart failure without diagnosed diabetes were screened with a standard 2 hour 75 gram oral glucose tolerance test following an overnight fast to determine glucose tolerance status (2 glucose value for IGT and DM were 140 and 200 mg/dl, respectively). Augmentation index (agph75) and carotid-radial pulse wave velocity (PWV) were obtained by applanation tonometry (Sphygmocor).

Results: 24/71 patients had normoglycemia (63% women, average age 55.9), 34/71 had IGT (67% women, average age 59.6), and 10/71 had diabetes (90% women, average age 65) with average BMI of 28.4, 27.2, and 29.7, respectively. Arterial stiffness or AGPH 75, was not different between patients with IGT and diabetes and were combined. There was a significant 30% increase in stiffness between patients with normoglycemia and IGT/DM ($15.3 \pm 9.4\%$ versus $21.04 \pm 9.4\%$, p-value = 0.017). AGPH75 was also significantly higher in patients with impaired glucose tolerance compared to normal. Linear regression analysis showed that glucose tolerance status was a significant and independent predictor of arterial vascular stiffness.

Conclusion:

Arterial stiffness measured by applanation tonometry is one of several noninvasive methods to identify individuals at risk for cardiovascular events. Our data indicate that patients diagnosed with IGT already have significant arterial stiffness. Therefore, therapies targeting the stage of impaired glucose tolerance is likely to impact vascular health. Whether interventions to control hyperglycemia will significantly reduce the burdens of congestive heart failure requires further study.

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3. Meguro T, Nagatomo Y, Nagae A, Seki C, Kondou N, Shibata M, et al. Elevated arterial stiffness evaluated by brachial-ankle pulse wave velocity is deleterious for the prognosis of patients with heart failure. *Circ J* 2009; 73(4):673-80.

Nothing to Disclose: PEO, JL, HK, ET, LS, JK, MAB

Pub # P2-561

Session Information POSTER SESSION: CLINICAL - Diabetes: Cardiometabolic Risk (1:30 PM-3:30 PM)

Title Short-Term Walnut Consumption Does Not Affect Markers of Inflammation and Vascular Injury in Obese Humans with the Metabolic Syndrome

Author String KN Aronis, F Magkos, MT Vamvini, LL Sweeney, A Brennan, CS Mantzoros
Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA; Harvard School of Public Health, Boston, MA; Boston VA Healthcare System, Boston, MA; Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA; Tolerx Inc, Cambridge, MA

Body
Introduction: Increased consumption of highly processed foods and reduced consumption of whole grains and nuts has been associated with elevated cardiovascular disease risk. Habitual nut consumption, on the other hand, has been consistently associated with reduced cardiovascular disease risk. However, the mechanisms responsible for the beneficial effect of dietary walnuts are not entirely clear. Limited evidence indicates that relatively long-term walnut consumption improves endothelial function in hypercholesterolemic patients, an effect possibly linked to improvements in several inflammatory, oxidation, and vascular injury biomarkers. In fact, both the improvement in endothelial function and antioxidant status manifest acutely, i.e., after just a single walnut-containing meal. However there is no evidence to date regarding the short-term effects of dietary walnuts on circulating markers of inflammation and vascular injury in humans.
Methods: We therefore evaluated the effects of short-term (4 days) walnut consumption on biomarkers of inflammation and vascular injury in 15 obese subjects, with BMI [ge] 30 kg/m², aged 40-75 years old, with metabolic syndrome (2006 International Diabetes Foundation criteria) in a randomized, double-blinded, placebo-controlled, crossover study. Subjects were randomized to isocaloric diets with liquid snacks with and without walnuts and were fed these diets as inpatients for 4 days. Both diets had the same macronutrient composition. After a wash-out period of one month, they completed the opposite arm of the study. Blood samples were collected on the first and last day of each admission, following a 12-hour fast. Cytokines and vascular injury biomarkers were measured using commercially available electro-chemi-luminescent assays (Meso Scale Discovery, Gaithersburg, MD).
Results: We found no significant differences between the walnut and the placebo diets on C-reactive protein, serum amyloid A, intercellular adhesion molecule 1, vascular cell adhesion protein 1, interleukin 6, interleukin 8, and tumor necrosis factor alpha concentrations in serum.
Conclusions: We conclude that short-term dietary consumption of walnuts does not affect levels of circulating inflammatory markers, cytokine and vascular injury markers, in obese humans with the metabolic syndrome.

Sources of Research Support: Grant Number M01-RR-01032-328840 to the Harvard Clinical and Translational Science Center, from the National Center for Research Resources. The Mantzoros Laboratory is also supported by the National Institute of Diabetes and Digestive and Kidney Diseases (grants DK058785, DK079929 and DK081913), the National Institute on Aging (grant AG032030) and the California Walnut Commission.

Disclosures: AB: Employee, Tolerx, Inc. Nothing to Disclose: KNA, FM, MTV, LLS, CSM

Pub # P2-562

Session Information POSTER SESSION: CLINICAL - Diabetes: Cardiometabolic Risk (1:30 PM-3:30 PM)

Title Albumin Concentration in Random Spot Urine Predicts Diabetic Nephropathy, Cardiovascular Events, and Mortality in Patients with Type 2 Diabetes

Author String LV Viana, JL Gross, JL Camargo, EPC Costa Rocha, MJ Azevedo
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Body Microalbuminuria is risk factor for diabetic nephropathy (DN), cardiovascular (CV) disease, and death. A random spot urine collection, using urinary albumin:creatinine ratio (ACR), has been recommended as the preferred sample for the screening of microalbuminuria. However, data on the predictive role of albuminuria is based mainly on 24h urine collections and few studies assessed the role of spot urine, especially urinary albumin concentration (UAC). The aim of this study was to analyze the predictive value of UAC for DN, cardiovascular events, and death as compared to ACR in a random urine spot. In this cohort, urinary albumin (immunoturbidimetry) was measured as 24h urinary albumin excretion (UAE) and, in random spot urine, UAC and ACR. Primary outcomes were: 1) DN, evaluated as a composite outcome [macroalbuminuria and/or decreased glomerular filtration rate (GFR) <60 ml/min/1.73m²]; 2) new cardiovascular events; and 3) death. A ROC curve was constructed to determine the cutoff point of UAC for prediction of DN. Kaplan-Meier curves and Multivariate Cox proportional hazards models estimated the end-of-study outcomes according each baseline urinary albumin measurement. A total of 199 type 2 diabetic patients, aged 59.9 ± 9.9 years, were followed for 6.1 ± 2.7 years. UAC [ge]14 mg/l predicted DN and the prediction for this and other outcomes was compared to the traditional microalbuminuria cutoffs points, ACR [ge]30 mg/g and UAE [ge] 30 mg/24h. The outcomes frequency was: DN=31.7% (23.5% decreased GFR, 13.6% macroalbuminuria); new cardiovascular events=26.4%; and death=8.50%. Cox analyses (hazard ratio [HR]; 95%CI) revealed that UAC [ge]14 mg/l increased 4.30 times the risk for DN composite outcome (2.22-8.32; P<0.001), 3.25 for cardiovascular events (1.43-7.38; P=0.005), and 5.51 for death (1.16-26.22; P=0.032). Corresponding HRs for ACR [ge]30 mg/g were: DN composite outcome 4.67(2.34-9.34; P<0.001), cardiovascular events 2.89 (1.29-6.45; P=0.009), and death 5.07(1.01-24.88; P = 0.049). HRs for UAE [ge]30 mg/24h were: DN composite outcome 6.76(3.32-13.77; P <0.001), cardiovascular events 2.20(2.08-2.49; P=0.030), and death 2.47(0.72-8.42; P=0.150). In conclusion, random UAC [ge]14 mg/l predicted DN, cardiovascular events, and mortality, likewise ACR. UAC may be used to assess renal and cardiovascular risks in patients with type 2 diabetes.

Sources of Research Support: Partially supported by FAPERGS-PRONEX, MCT/CNPq, FIPE-HCPA, and CAPES.

Nothing to Disclose: LVV, JLG, JLC, EPCCR, MJA

Pub # P2-563

Session Information POSTER SESSION: CLINICAL - Diabetes: Cardiometabolic Risk (1:30 PM-3:30 PM)

Title Metabolic Syndrome and Central Fat Distribution Are Related to Lower Serum Osteocalcin Concentrations

Author String K Magalhaes, M Magalhaes, E Diniz, C Lucena, F Bandeira
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Body Objective: Some studies have suggested a role of the skeleton in the regulation of glucose and fat metabolism by osteocalcin, a protein secreted by osteoblasts during bone formation. The aim of this study was to evaluate the association of serum osteocalcin with metabolic syndrome (MS) in men and premenopausal women. To assess whether this association would not only be a reflection of an association between bone turnover and MS, the serum values of C- telopeptide (CTX), a marker of bone resorption, were also measured. Methods: We evaluated 14 middle-aged men and 44 premenopausal women. The exclusion criteria were: menopause, diagnosis of malignancy, elevated serum creatinine, uncontrolled thyroid disease, liver disease or conditions which interfere in bone metabolism (hyperparathyroidism, Paget's disease of bone, osteomalacia, the use of bisphosphonates, calcitonin, estrogens, testosterone and glitazones). MS was defined according to the International Diabetes Federation criteria. Anthropometric data were collected and serum osteocalcin, serum CTX, fasting plasma glucose (FPG) and lipid profile were measured. Results: Mean age was $41,07 \pm 8,4$ year; and did not differ between patients with and without MS. Mean serum osteocalcin was significantly lower in patients with MS ($11,18 \pm 4,62$ vs $15,09 \pm 5,05$, $p = 0,003$) and decreased significantly as the number of MS criteria increased ($15,72 \pm 5,25$ in patients with one criteria, $12,24 \pm 4,00$ in patients with two or three criteria and $10,73 \pm 5,61$ in patients with four or five criteria, $p = 0,012$). There were no significant differences in serum CTX between the two groups. Serum Osteocalcin was lower in patients with body mass index (BMI) $[ge] 25 \text{ kg/m}^2$ ($p = 0,038$), FPG $[ge] 100 \text{ mg/dL}$ ($p = 0,024$), in hypertensive ($p = 0,013$) and diabetic patients ($p = 0,036$), and was inversely associated with BMI ($p = 0,024$), waist circumference ($p = 0,024$), FPG ($p = 0,007$) and systolic blood pressure (SBP) ($p = 0,037$). Conclusion: Our data showed that lower serum osteocalcin was associated with the presence of MS and that osteocalcin was inversely associated with BMI, waist circumference, FPG and SBP. These findings support the hypothesis that osteocalcin plays a role in the regulation of glucose and fat metabolism and may be involved in the development of metabolic syndrome.

Nothing to Disclose: KM, MM, ED, CL, FB

Pub # P2-564

Session Information POSTER SESSION: CLINICAL - Diabetes: Cardiometabolic Risk (1:30 PM-3:30 PM)

Title Insulin Resistance Is Neither Necessary nor Required for the Development of Metabolic Syndrome

Author String R Karnchanasorn, H-Y Ou, L-M Chuang, KC Chiu
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Body Metabolic syndrome (MetS) is described as a cluster of interrelated risk factors for cardiovascular disease (CVD). Patients with MetS carry 2-fold risk of CVD. Insulin resistance (IR) has been proposed as one of the potential causation of this syndrome. We examined the role of IR in MetS.
We analyzed the data from participants age 1-84 years old (y/o) in the National Health and Nutrition Examination Survey (NHANES) 1999 - 2000, a cross sectional survey of the U.S. population. Only the subjects with fasting glucose and insulin and the components of MetS were included in this analysis. The sample consists of 911 Non-Hispanic whites (NHW), 398 Non-Hispanic blacks (NHB), and 595 Mexican Americans (MA). We quantified IR by using the homeostatic model assessment from fasting glucose and insulin concentrations. MetS is defined based on the revised Adult Treatment Panel III. Based on IR, they were divided into insulin resistant (highest tertile) and non-insulin resistant (middle and lowest tertiles). We also consider potential confounders: age, gender, BMI, education, poverty index, physical activity, alcohol use, and smoking.
Among all ethnicities, IR was a risk factor for MetS ($P < 0.0001$) and remained after adjustment for the covariates ($P < 0.0001$). However, there was substantial number of IR subjects who did not have MetS: 35% in NHW, 34% in NHB, and 31% in MA. Moreover, there were 21% of NHW, 15% of NHB, and 17% of MA with MetS who were not in the IR category.
We also examined the association of IR with various components of MetS: waist circumference, triglyceride, HDL cholesterol, systolic and diastolic blood pressure. The associations that persisted among 3 ethnic groups were triglycerides and HDL cholesterol (Adjusted $P < 0.01$). The association of IR with waist circumference was only observed in NHW and HNB (adjusted $P < 0.0001$), but not in MA. The association of IR with systolic blood pressure was only observed in MA (adjusted $P = 0.003$), but not in NHW or NHB. No association of IR with diastolic blood pressure was observed in any ethnic group.
In the present study, we observed that not all IR subjects had MetS and not all subjects with MetS were IR. Furthermore, the associations of IR with each component of MetS were not persistent among all three ethnic groups. Our observation indicates that IR is neither necessary nor required for the development of MetS.

Nothing to Disclose: RK, H-YO, L-MC, KCC

Pub # P2-565

Session Information POSTER SESSION: CLINICAL - Diabetes: Cardiometabolic Risk (1:30 PM-3:30 PM)

Title Hypertension and Hyperglycemia Are the Main Determinants behind the Association of Metabolic Syndrome with Chronic Kidney Disease in Subjects with Different Degrees of Glucose Tolerance

Author String AL Smith, BG Borges Fortes, AD Americo, V Piccoli, A Fabbrin, SP Garcia, B Jaeger, EP Rocha, G Ghisleni, LH Canani, F Gerchman
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Body Accumulating evidence supports that metabolic syndrome (MS) has an important role in the development of chronic kidney disease (CKD) (1). We examined this relationship and its possible determinants in a cross-sectional study of subjects (n=118; females 78.8%; age 52.9±11.2; mean±SD) assisted at the Pre-diabetes (Pre-DM) and Endocrine clinics of a university hospital. Subjects were submitted to a 2-h 75g oral glucose tolerance test and classified in normal glucose tolerance (NGT; n=30), Pre-DM (n=60) and diabetes (DM; n=28). MS was defined according to the new Joint Scientific Statement Harmonization definition as the presence of 3 out of 5 of the following criteria: hypertension, low HDL-cholesterol, high triglyceride levels, hyperglycemia and high waist circumference (2). Glomerular filtration rate (GFR) was estimated by the CKD Epidemiology Collaboration (CKD-EPI) equation, insulin resistance by HOMA-IR, renal damage by microalbuminuria and endothelial dysfunction by fibrinogen. GFR was lower in subjects with MS than those without MS (102.6±14.3 vs 93.0±18.7 ml/min per 1.73m²; P=0.027). GFR decreased with the increasing number of MS criteria (0 or 1 criteria 104.9±14.7 vs 2 criteria 101.5±14.5 vs 3 or 4 criteria 96.3±17.4 vs 5 criteria 83.3±19.6 ml/min per 1.73m²; P=0.002). Among the MS criteria, GFR was lower in those with hypertension (P=0.004) and hyperglycemia (P=0.005), it was not statistically lower in subjects with hypertriglyceridemia (P=0.064), and it was similar in those with low HDL (P=0.196) and elevated waist circumference (P=0.539) than those without each one of these criteria. Systolic arterial blood pressure (SBP) was inversely related to GFR (r=-0.324; P=0.001) whereas diastolic arterial blood pressure (DBP), fasting and 2-h plasma glucoses, waist circumference, HDL cholesterol, triglycerides, HOMA-IR, microalbuminuria and fibrinogen were not related to GFR. By multiple linear regression analysis, GFR was associated with increased fasting plasma glucose (R=0.197; P=0.038) and SBP (R=-0.344; P<0.001) while it was not associated with waist circumference, triglycerides or HDL cholesterol. The previously described association between MS and CKD was confirmed in our study using the new harmonizing criteria for MS. This relationship was not related with insulin resistance, renal damage or endothelial dysfunction and it was mostly determined by abnormalities in blood pressure homeostasis and hyperglycemia.

(1) Sowers JR, *Kidney International* 2007; 71: 719

(2) Alberti KG et al., *Circulation*; 2009; 120: 1640

Sources of Research Support: Brazilian National Research Council (CNPq); Coordination of Higher Education and Graduate Training (CAPES); International Scholarship Program of the Endocrine Society; Hospital de Clinicas de Porto Alegre Research and Events Incentive Funds (FIPE-HCPA); Rio Grande do Sul Research Foundation (FAPERGS).

Nothing to Disclose: ALS, BGBF, ADA, VP, AF, SPG, BJ, EPR, GG, LHC, FG

Pub # P2-566

Session Information POSTER SESSION: CLINICAL - Diabetes: Cardiometabolic Risk (1:30 PM-3:30 PM)

Title Monocyte Chemotactic Protein-1 Levels Are Significantly Higher Postprandially in Patients with Type 2 Diabetes Than in Matched Controls

Author String WM Widdowson, AM McGowan, G Boran, J Gibney
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Body Introduction: Post-prandial metabolic abnormalities, most notably hyperlipidaemia and inflammation, are known to play a role in the development of atherosclerosis in Type 2 diabetes. We performed a study to investigate the post-prandial levels of inflammatory cytokines in patients with type 2 diabetes compared to matched controls.

Methods: Patients were randomly recruited from the routine hospital gastroscopy list and underwent a standardised mixed meal test (total energy 940 kcal, carbohydrate 140g, fat 36g), with serum samples taken fasting and at 2 hourly intervals post-prandially. Measurements included standard anthropomorphic parameters, as well as biochemical, glucometabolic, and lipid profiles. Inflammatory cytokines were analysed using a Randox array ELISA. Patients with type 2 diabetes were age, gender and BMI matched to non-diabetic controls, and compared using independent samples t tests and Pearsons correlation coefficient on SPSS.

Results: Seven matched pairs were included in the analysis (mean age 50.4 years, BMI 34.9 kg/m²). No significant differences were detected between groups for insulin AUC, HOMA-IR, or triglyceride AUC. Significant increases were detected post-prandially in white cell and neutrophil count, triglyceride and apolipoprotein B48 levels in the whole group, with no significant difference between groups. Analysis of ten inflammatory cytokines revealed a significantly raised level of monocyte chemotactic protein-1 (MCP-1) in diabetic subjects vs controls (2224.5 ± 447.6 vs 1762.2 ± 248.2 , $p < 0.05$). MCP-1 levels correlated strongly with HOMA-IR ($r = 0.606$, $p < 0.05$) and triglyceride AUC ($r = 0.625$, $p < 0.05$) across both groups. Levels of pro-inflammatory cytokines IL-4 and IL-6 were also raised post-prandially across the whole cohort (1.44 vs 1.54 pg/mL and 2.36 vs 5.98 pg/mL respectively; $p < 0.01$), but were not significantly different between groups.

Conclusion: MCP-1 is shown to be significantly elevated in the post-prandial period in patients with Type 2 diabetes versus matched controls. MCP-1 has previously been shown to be involved in the pathogenesis of atherosclerosis at multiple levels, and has also been implicated in the pathogenesis of insulin resistance. The current finding of increased post-prandial levels in patients with type 2 diabetes is novel in the literature, may be involved in either of the two mechanisms mentioned above, and warrants further investigation.

Sources of Research Support: Meath Foundation.

Nothing to Disclose: WMW, AMM, GB, JG

Pub # P2-567

Session Information POSTER SESSION: CLINICAL - Diabetes: Cardiometabolic Risk (1:30 PM-3:30 PM)

Title Effects of Add-On Nebivolol on Glucose Parameters in Hypertensive Patients with Metabolic Syndrome

Author String P Deedwania, L Brener, W Chen, J Shea, J Whalen
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Body Many patients with hypertension have metabolic syndrome (MetS), and treatment with certain β -blockers may worsen glycemic measures. Nebivolol (Neb) is a vasodilating β -blocker. This post hoc analysis examined the effects of Neb on glycemic parameters and safety in patients with MetS from a Neb trial that recruited patients with hypertension and prediabetes. Patients with impaired fasting plasma glucose (FPG: 100-125 mg/dL) and/or elevated 2-hour post-oral glucose tolerance test (OGTT: 140-199 mg/dL) at screening received lisinopril (10 mg/d) or losartan (50 mg/d) during a 4-wk run-in period. Those with DBP 90-110 mmHg at the end of run-in (baseline) were randomized (2:2:1) to double-blind add-on treatment (Neb, 5-40 mg/d [n=223]; HCTZ, 12.5-25 mg/d [n=212]; placebo [Pbo; n=102]; total: N=537), titrated to achieve SBP/DBP [e]130/80 mmHg over 12 weeks. Outcomes included mean [95%CI] changes in FPG, OGTT, HOMA-IR, and fasting plasma insulin (FPI). A total of 375 patients had MetS at baseline: (Pbo, n=67; 5-20 mg/d Neb [5-20Neb], n=62; 40 mg/d Neb [40Neb], n=98; HCTZ, n=148). At week 12, change from baseline (mg/dL) in FPG was 0.5 [-2.5, 3.6] for Pbo, -2.7 [-5.6, 0.2] for 5-20Neb, 0.5 [-2.6, 3.6] for 40Neb, and 4.1 [1.5, 6.8] for HCTZ. For AUC of OGTT, change from baseline (mg/dL) was -0.6 [-7.2, 6.0] for Pbo, -9.6 [-17.0, 2.2] for 5-20Neb, 1.8 [-3.1, 6.6] for 40Neb, and 6.9 [2.1, 11.6] for HCTZ. Change from baseline in HOMA-IR was 0.0 [-1.1, 1.1] for Pbo, -0.6 [-1.6, 0.4] for 5-20Neb, 0.8 [-0.8, 2.4] for 40Neb, and 1.6 [0.4, 2.8] for HCTZ. Change from baseline (mU/L) in FPI was 2.4 [-24.1, 28.9] for Pbo, -15.9 [-45.2, 13.3] for 5-20Neb, 7.6 [-20.4, 35.8] for 40Neb, and 29.5 [3.0, 56.1] for HCTZ. In the entire MetS sample, the most common adverse events (AEs) were headache, upper respiratory tract infection, diarrhea, back pain, bradycardia, fatigue, and dizziness. There were 3 AEs in the Neb group (all dosages) that occurred at a rate [ge]3% and more often than in the Pbo group: bradycardia, diarrhea, and back pain; for HCTZ, such AEs were back pain, fatigue, and dizziness. Rates of serious AEs were 3.0% (Pbo), 3.1% (Neb), and 3.4% (HCTZ). These data suggest that nebivolol, added to an ACEI or ARB, is well tolerated and has little or no effect on glucose metabolism in hypertensive patients with metabolic syndrome.

Disclosures: PD: Consultant, Novartis Pharmaceuticals; Forest; GlaxoSmithKline; Pfizer, Inc.; Speaker Bureau Member, Novartis Pharmaceuticals; Forest; GlaxoSmithKline; Pfizer, Inc. LB: Employee, Forest Research Institute. WC: Employee, Forest Research Institute. JS: Employee, Forest Research Institute. JW: Employee, Forest Research Institute.

Pub # P2-568

Session Information POSTER SESSION: CLINICAL - Diabetes: Cardiometabolic Risk (1:30 PM-3:30 PM)

Title Serum Cystatin C as Well as Urinary Albumin-Creatinine Ratio Is Associated with Carotid Artery Intima-Media Thickness (IMT) in Korean Type 2 Diabetic Patients

Author String K-S Park, S-K Kim, S-W Park, Y-W Cho
CHA Bundang Medical Center, CHA University, Seongnam, Korea

Body
Background: There are several markers of renal function such as estimated glomerular filtration rate (GFR) calculated by serum creatinine, serum cystatin C and urinary albumin-creatinine ratio (ACR). On the other hand, carotid artery intima-media thickness (IMT) has been used for early detection of atherosclerosis. Recently, a few studies showed relationships between markers of renal function and carotid artery IMT, but the results were inconsistent. The aim of this study is to compare serum cystatin C and urinary ACR as risk factors for atherosclerosis in Korean type 2 diabetic patients.
Methods: This study was performed in 310 type 2 diabetic patients, who were evaluated by serum cystatin C, urinary ACR (both divided into tertiles) and carotid artery IMT with other clinical characteristics. Estimated GFR was calculated using the modification of diet in renal disease (MDRD) equation. The carotid artery IMT was assessed by B-mode ultrasonography.
Results: There were no differences in the proportion of gender, obesity, smoking and dyslipidemic subjects across tertiles of serum cystatin C. Higher levels of serum cystatin C were associated with older age, longer duration of diabetes, lower HDL-cholesterol level and increased carotid artery mean IMT (P for trend <0.001, 0.003, 0.001 and <0.001, respectively). Participants with higher urinary ACR also had longer duration of diabetes and increased carotid artery mean IMT (P for trend 0.042 and 0.044, respectively), but mean age and serum cholesterol levels did not significantly differ among tertiles of urinary ACR. In multivariate analyses adjusted for traditional atherosclerotic risk factors, carotid artery mean IMT was strongly associated with urinary ACR and serum cystatin C (P for trend 0.038 and 0.045, respectively), but not with estimated GFR (P=0.866).
Conclusion: In this study, we showed relationships between carotid artery IMT and markers of renal function except estimated GFR beyond traditional atherosclerotic risk factors in Korean type 2 diabetic patients. Measurement of these renal parameters might be important for early detection of atherosclerosis as well as renal insufficiency in type 2 diabetic patients.

Nothing to Disclose: K-SP, S-KK, S-WP, Y-WC

Pub # P2-569

Session Information POSTER SESSION: CLINICAL - Diabetes: Cardiometabolic Risk (1:30 PM-3:30 PM)

Title Acute Hyperglycemia and Cardiovascular Risk in Transient Ischemic Attack Patients

Author String T Barrera, M Marcelli, T Kent
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Body **Objective:** To evaluate whether acute hyperglycemia in patients who present with transient ischemic attack increases the incidence of cardiovascular outcomes or mortality as compared to normoglycemic patients. **Methods:** This is an interim analysis of a retrospective study of patients who presented to the hospital with TIA between January 2000 and December 2008. Inclusion criteria included diagnosis of TIA with symptoms on day of presentation and no longer than 24 hours. Glucose values recorded on date of admission were assessed and those that were ≥ 126 mg/dL were considered hyperglycemic irrespective of diabetes history. Cardiovascular risk factors such as atrial fibrillation, hyperlipidemia (LDL), hypertension and obesity were accounted for. Patients were also assessed for any subsequent events such as death, ACS, stroke, TIA, or MI. Differences between subjects who later developed an event and those that did not were analyzed by ANOVA (continuous variables) or X^2 (categorical variables). Kaplan- Meier analysis was used to determine difference in time-to-event by glycemic state (normoglycemic vs hyperglycemia). **Results:** Out of 73 patients that were included in the study, 28 patients had known history of diabetes. 15 patients experienced subsequent events after their TIA of which 10 of these patients did not have history of diabetes. ANOVA analysis did not show any statistical significance for blood glucose ($P=0.256$), BMI ($P=0.477$), LDL cholesterol ($P=0.469$). In relation to cardiovascular events, no statistically significant relationships were established when looking at the following variables: glucose ≥ 126 ($P=0.661$), hypertension ($P=0.126$) or atrial fibrillation ($P=0.655$). Kaplan-Meier analysis did not show any significant differences in time to event by glycemic status. **Conclusion:** With the current data, no significant association between acute hyperglycemia and subsequent cardiovascular events or mortality has been established. Cardiovascular risk factors as aforementioned have not demonstrated any influence on outcomes. One limiting factor in this interim analysis is that the sample size is small and this limits the results. To achieve power and statistical significance we will need to recruit a total of 146 patients in which 73 are assigned to either hyperglycemic or normoglycemic, respectively. We hope to clearly identify any association that may exist between acute hyperglycemia and subsequent cardiovascular events and/or death.

Nothing to Disclose: TB, MM, TK

Pub # P2-570

Session Information POSTER SESSION: CLINICAL - Diabetes: Cardiometabolic Risk (1:30 PM-3:30 PM)

Title Cardiovascular Risk Factor Management in Diabetic South Asians and Rate of Myocardial Infarction

Author String R Ahrafi, M Biswas
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Body

Background
Earlier studies have demonstrated that rates of cardiovascular disease are up to 5 times higher in persons of South Asian origin when compared with age matched white Caucasians and partly attributable to a higher prevalence of type 2 diabetes. Recent studies have shown that with improved risk factor management, MI incidence in South Asians may approach that of white Caucasians.
We conducted a retrospective study of myocardial infarction rates in locally residing South Asians and cardiovascular risk factor management in South Asians attending our hospital diabetic clinic.

Methods
From our clinical coding department, we identified the total number of South Asians admitted to hospitals within the Aneurin Bevan Health Trust with a diagnosis of 'myocardial infarction' (MI) between April 2008 and April 2010.
MI consisted of Non-ST elevation (NSTEMI) and ST elevation (STEMI), defined as a Troponin I level above the laboratory specific reference limit of 0.08 mg/L in association with ECG changes and/or chest pain. Clinical records of South Asians attending our hospital diabetic clinic between May 2009 and 2010 were subsequently examined.

Results:
Out of a total of 1853 myocardial infarctions, 21 patients were of South Asian origin and of these only 6 were diabetic, all type 2. 8/21 had NSTEMI, managed medically, 7/21 had triple vessel surgical disease and 6/21 required PCI (1/21 required PCI for STEMI).
82 South Asians attending the hospital diabetic clinic were studied of which 97% had type 2 diabetes, 46% were male and 51% were insulin requiring. Of these patients, 77% were receiving ACE inhibitors or AT 2 receptor antagonists and 73% were treated with statins. The mean HbA1c was 8.75% (SD1.64), mean systolic blood pressure was 135.24 (SD 19.24) and mean total cholesterol 3.95 mmol/l (SD 0.85).

Conclusion
According to self reported ethnicity data from the census, 0.9% of people residing in South East Wales reported themselves to be of South Asian origin (5400 out of 600 000) Our data reveal that MI incidence in South Asians is slightly higher than in White Caucasians 0.004 /year vs 0.003/year respectively, though ethnic differences in our population are less than previously reported. Also in contrast to previous studies, only 28% of South Asians admitted with MI were diabetic. Encouragingly, cardiovascular risk modification therapies are being utilized by the majority of South Asians attending the hospital diabetic clinic.

Nothing to Disclose: RA, MB

Pub # P2-571

Session Information POSTER SESSION: CLINICAL - Diabetes: Cardiometabolic Risk (1:30 PM-3:30 PM)

Title Close Association of Serum Fibroblast Growth Factor (FGF) 23, but Not 25(OH)Vitamin D₃, with Diabetic Nephropathy in Type 2 Diabetic Subjects

Author String M Murata, A Aoki, T Asano, A Ikoma, M Sasaki, M Kawakami, S-e Ishikawa
Jichi Medical University Saitama Medical Center, Saitama, Japan

Body **Objectives:** The present study was undertaken to determine alterations in serum fibroblast growth factor 23 (FGF-23) and 25(OH)Vitamin D₃ levels in subjects with diabetic nephropathy and atherosclerotic disorders. **Methods:** 247 type 2 diabetic patients were enrolled in the present study. They were 149 males and 98 females, with the ages of 64.5±10.2 years. Serum levels of FGF-23 and 25(OH)Vitamin D₃ were measured by methods of ELISA. Serum creatinine, estimated glomerular filtration rate (eGFR) and urinary albumin and/or protein excretion were determined for classification of diabetic nephropathy. Flow-mediated dilatation (FMD), intima-media thickness (IMT) and calcification of carotid artery were examined for atherosclerosis. **Results:** Serum FGF-23 levels were 35.7±1.1 pg/ml, ranging from 10.0 to 148.0 pg/ml. Serum 25(OH)Vitamin D₃ levels were 86.1±2.5 nmol/l, ranging from 28.3 to 239.3 nmol/l. We divided the diabetic subjects into two groups by the median level of FGF-23 (31.6 pg/ml). In the subjects of serum FGF-23 level more than 31.6 pg/ml (high FGF-23; n=124), BMI was significantly higher (25.4±3.9 vs. 23.6±3.5, p<0.001) and eGFR was significantly less (72.2±2.2 vs. 82.2±1.7 ml/min/1.73m², p<0.001) than those in low FGF-23 group. In single regression analysis, serum FGF-23 levels had a positive correlation with BMI (r=0.25, p<0.001), and a negative correlation with eGFR (r=-0.30, p<0.001). Serum FGF-23 levels were gradually increased according to the progression of diabetic nephropathy (p value in trend test: <0.001). Its elevation was significantly greater in the diabetic subjects with stage 4 than those with stages 1, 2 and 3A. There were no differences in FMD, IMT and vascular calcification between the two groups of high and low FGF-23. Also, we divided the subjects by median of 25(OH)Vitamin D₃ (79.2 nmol/l), and evaluated the differences between the two groups. However, there was no difference in any parameter at all. **Conclusions:** These findings indicate that serum FGF-23 levels increase in association with progression of diabetic nephropathy, but not with atherosclerosis, in type 2 diabetic subjects.

Nothing to Disclose: MM, AA, TA, AI, MS, MK, S-EI

Pub # P2-572

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Steroidogenesis & Its Disorders (1:30 PM-3:30 PM)

Title The Rapid Intermediate P450_{scc} in the Intermembrane Space Regulates Steroidogenesis

Author String M Prasad, HS Bose
Mercer University School of Medicine and Memorial University Medical Center, Savannah, GA

Body Mitochondrial proteins are usually synthesized in the cytosolic endoplasmic reticulum and then imported into one of the four different mitochondrial subcompartments: outer mitochondrial membrane (OMM), inner mitochondrial membrane (IMM), inner mitochondrial space (IMS) and matrix. Cytochrome P450 side-chain cleavage enzyme (P450_{scc}), a key component of the mitochondrial P450 complex, cleaves the side chain from cholesterol to form the first steroid in the tissue- pregnenolone. Thus the active participation of the P450_{scc} is crucial for reproduction, sexual differentiation and survival of all species. The import of in vitro-translated, ³⁵S-labeled precursor SCC into mitochondria isolated from mammalian cells and adrenal tissues resulted in three distinctly-sized labeled bands; however, import into mitochondria isolated from yeast produced two bands. We report that translocation of cytochrome P450_{scc} into mammalian mitochondria proceeds via a translocation intermediate (iSCC) lodged between the two mitochondrial membranes and then translocated as mature form (mSCC) at the matrix. To refine the localization of iSCC and mSCC in the mitochondria, we isolated the OMM, IMM and matrix by digitonin and lubrol. As expected, pSCC and iSCC were present in OMM fraction however most of the mSCC was present in the matrix fraction. The import in presence of various metabolic inhibitors: oligomycin (OLIGO), valinomycin (VAL), and carboxyatractiloid (CAT) suggests that the processing of iSCC to mSCC occurs at the IMS and requires a potential across the IMM. We evaluated the effects of the various inhibitors on metabolic activity in mitochondria preincubating with ¹⁴C-Cholesterol. SCC exerts its activity during transient state. The active iSCC is rapidly converted to mSCC which accumulates in matrix and so a rapid degradation of mSCC is essential to bring another pool of iSCC. This is the first report describing the mechanism by which SCC can quickly metabolize a large pool of cholesterol and thereby contribute to the regulation of steroid hormone serum concentrations.

Nothing to Disclose: MP, HSB

Pub # P2-573

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Steroidogenesis & Its Disorders (1:30 PM-3:30 PM)

Title Absence of 11 β -HSD2 Specifically within the Fetal Brain Alters Adult [lsquo]Depressive[rsquo] Behavior

Author String CS Wyrwoll, JR Seckl, MC Holmes
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Body Maternal stress and consequent fetal glucocorticoid overexposure increases offspring susceptibility to neuropsychiatric disorders. Fetal brain exposure to glucocorticoids is regulated by 11 β -hydroxysteroid dehydrogenase type 2 (11 β -HSD2) which inactivates glucocorticoids in the placenta and fetal brain. We have shown that 11 β -HSD2 $^{-/-}$ offspring generated by heterozygous matings exhibit altered placental development and function, decreased birth weight, delayed neurodevelopment and increased anxiety and depressive-like behaviour as adults. This raises the question as to whether it is placental or fetal brain 11 β -HSD2 that underpins programmed outcomes? A knockout of 11 β -HSD2 specifically within the brain during development was created by crossing NestinCre mice with floxed 11 β -HSD2 mice (HSD2flx/flx). 11 β -HSD2 activity in fetal tissue and placenta was measured at E12.5, neurodevelopmental landmarks assessed and adult behaviour characterised. Brain-specific reduction in 11 β -HSD2 activity was confirmed at E12.5 in the fetal heads of NestinCre.HSD2flx/flx mice in comparison to HSD2flx/flx (2.01 \pm 0.24 and 10.13 \pm 1.69 nmol A/mg/h resp, P<0.01). Birth weight, negative geotaxis and eye opening, markers of neurodevelopment, were unaltered. Anxiety was assessed by elevated plus maze and open field in unstressed and acutely stressed adult offspring and was unchanged. However depressive-like behaviour in the tail suspension test was increased in mice with brain-specific deletion of 11 β -HSD2 with NestinCre.HSD2flx/flx spending a greater percentage of time hanging in comparison to HSD2flx/flx mice (68 and 53% respectively, P<0.05). Depressive-like behaviour was also exhibited in novelty-induced hypophagia with the NestinCre.HSD2flx/flx mice having a reduced latency to feed in comparison to the HSD2flx/flx mice (53 \pm 8s and 28 \pm 4s resp, P=0.01). Our data suggest that fetal brain 11 β -HSD2 impacts specifically on depressive-like behaviours, but that broader anxiety-related and neurodevelopmental effects are likely to relate to indirect effects of 11 β -HSD2 in the placenta.

Sources of Research Support: The Wellcome Trust.

Nothing to Disclose: CSW, JRS, MCH

Pub # P2-574

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Steroidogenesis & Its Disorders (1:30 PM-3:30 PM)

Title A Potential Mechanism for Regulation of Aldosterone Production by Control of Translocation of Aldosterone Synthase into Mitochondria

Author String BP Adams, HS Bose
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Body Aldosterone synthase (AS) regulates blood volume by catalyzing production of aldosterone, a mineralocorticoid. Disorders in production of aldosterone can cause hypertension, a major factor in heart disease and stroke. Control of aldosterone production is regulated by the renin-angiotensin system in a promoter dependent fashion. Expression of AS and P450SCC are stimulated by Angiotensin II, an effect that takes 12 hours to detect and lasts 24 hours. We have found a promoter independent mechanism which could regulate aldosterone production. This mechanism involves the mitochondrial translocation and processing of AS. To study translocation, we incubated radiolabeled AS with mitochondria isolated from NCI-H295 cells and analyzed by SDS-PAGE. Translocated AS was cleaved, removing 24 amino acids from the N-terminus. We found that AS was cleaved in the mitochondrial matrix by mitochondrial processing peptidase (MPP). Production of aldosterone by AS was measured using a radio immunoassay (RIA). Translocation into mitochondria is required for maximal production of aldosterone, and AS is more active when residing in the intermembrane space (IMS). Increased translocation across the outer mitochondrial membrane would therefore result in higher aldosterone production, and vice versa. Once AS is cleaved by MPP in the mitochondrial matrix, it is rendered inactive. Therefore, slower translocation across the inner mitochondrial membrane (IMM) would increase production of aldosterone, while faster IMM translocation would deactivate AS more quickly. These results reveal a potential system for regulation of aldosterone production in the mitochondria that is both rapid and promoter independent.

Nothing to Disclose: BPA, HSB

Pub # P2-575

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Steroidogenesis & Its Disorders (1:30 PM-3:30 PM)

Title Dynamic Regulation of 11 β -Hydroxysteroid Dehydrogenase Type 1 in Neutrophils during an Inflammatory Response

Author String AE Coutinho, T Kipari, Z Zhang, C Esteves, JS Gilmour, J-F Cailhier, J Hughes, JR Seckl, JS Savill, KE Chapman
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Body Within cells, endogenous glucocorticoid action is enhanced by pre-receptor metabolism by 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1), which converts intrinsically inert cortisone (in rodents, 11 dehydrocorticosterone; 11-DHC) into active cortisol (in rodents, corticosterone; CORT). 11 β -HSD1 is highly expressed in immune cells elicited to the mouse peritoneum during thioglycollate (TG)-induced peritonitis and is down-regulated as the inflammation resolves. During sterile peritonitis, 11 β -HSD1-deficient mice show enhanced recruitment of inflammatory cells and delayed acquisition of macrophage phagocytic capacity. However, the key cells in which 11 β -HSD1 exerts these effects remain unknown. Here, we have identified neutrophils (CD11b⁺/Ly6G⁺/7/4⁺ cells) as the TG-recruited cells that most highly express 11 β -HSD1 and show regulation of 11 β -HSD1 in these cells during an inflammatory response. Flow cytometry assessment of peritoneal neutrophils showed that the Mean Fluorescent Intensity (MFI) of 11 β -HSD1, in comparison to 0h (300 \pm 15), was higher at 4h (6134 \pm 32) (p<0.001) and 24h (372 \pm 10) (p<0.05), but lower at 96h (131 \pm 3) (p<0.001) post TG injection. Blood neutrophils had higher 11 β -HSD1 MFI at 4h (938 \pm 40), 24h (1014.3 \pm 41) and 96h (1113 \pm 31) compared to 0h (619 \pm 16) (p<0.001). In bone marrow, 11 β -HSD1 MFI in neutrophils was only up-regulated at 24h (1099 \pm 46) in comparison to 0h (922 \pm 51) (p<0.05). Isolation of neutrophils with Ly6G-magnetic beads from total peritoneal cells 24h post TG injection, showed 6.5 fold higher 11 β -HSD1 mRNA levels in peritoneal neutrophils than in non-neutrophil cells (mainly monocytes/macrophages). 11 β -HSD1 activity in 24h TG neutrophils was higher than non-neutrophils (neutrophils; 45.0 \pm 1.6% vs non-neutrophils; 17.6 \pm 2.2% conversion of 11-DHC to CORT after 40min culture, p<0.001). Ablation of monocytes/macrophages by treatment of CD11b-diphtheria-toxin receptor transgenic mice with diphtheria toxin prior to TG injection had no significant effect on 11 β -HSD1 activity in peritoneal cells (controls; 21.5 \pm 2.1% vs DT-treated; 27.5 \pm 0.7% conversion of 11-DHC to CORT) consistent with neutrophils being the high 11 β -HSD1 expressing cell type at this time. These data suggest that neutrophils may play a role in the immunoregulatory effect of 11 β -HSD1 and that the earlier and more severe inflammatory response seen in 11 β -HSD1-deficient mice may be due to the lack of restraining effect of glucocorticoids in neutrophils.

Nothing to Disclose: AEC, TK, ZZ, CE, JSG, J-FC, JH, JRS, JSS, KEC

Pub # P2-576

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Steroidogenesis & Its Disorders (1:30 PM-3:30 PM)

Title Modulation of *CYP17A1* Gene Expression by miR-320

Author String LA Diver, S Wood, SM MacKenzie, JM Connell, E Davies
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Body
Introduction: The *CYP17A1* gene has been strongly implicated in the regulation of blood pressure and hypertension by recent genome-wide association studies. Its enzyme product functions both as a 17 α -hydroxylase and a 17,20 lyase in steroidogenesis. Recently, microRNAs have emerged as novel post-transcriptional regulators of gene expression and we have investigated their role in adrenal steroidogenesis. We have demonstrated that knockout of Dicer1, a protein required for microRNA action, significantly increases levels of *CYP17A1* mRNA levels in the H295R human adrenocortical cell line, implicating microRNA in this gene's regulation. Furthermore, our bioinformatic analysis predicts that the microRNA miR-320a directly targets adrenal *CYP17A1*. We therefore investigated whether miR-320a acts as a negative regulator of *CYP17A1*.
Methods: We transfected the H295R human adrenal cell line with miR-320 pre-miR or anti-miR molecules (50nM). The pre-miR is a synthetic microRNA that supplements endogenous miR-320 levels, while the anti-miR stimulates the degradation of endogenous miR-320. Scrambled pre-miRs and anti-miRs were used as controls. *CYP17A1* mRNA and miR-320 levels were measured by qRT-PCR at 48h post-transfection, using *GAPDH* as a housekeeping gene. Reactions were performed in triplicate with at least two biological replicates.
Results: *CYP17A1* mRNA levels were reduced from 100% in control cells to 35.3% \pm 3.99% with pre-miR transfection (p[le]0.01). Anti-miR transfection increased levels to 167.8% \pm 10.27% (p[le]0.05). miR-320 levels increased to 5469% \pm 1174% (p[le]0.05) with pre-miR transfection and fell to 0.36% \pm 0.33% (p[le]0.001) with anti-miR transfection, relative to 100% in control cells.
Conclusion: We have shown that miR-320a significantly reduces *CYP17A1* mRNA abundance in H295R cells. Furthermore, inhibition of miR-320a results in dramatically increased levels of *CYP17A1* transcripts. Such modulation of steroidogenic gene expression by microRNA is likely to result in major changes to steroid production. This offers the strongest available evidence that miR-320a has a major role in the modulation of adrenal steroidogenesis. This is supported by our previous studies showing miR-320a levels to be dysregulated in aldosterone-producing adenoma (APA) tissue and by our prediction of a miR-320a binding site in mRNA encoding the side-chain cleavage enzyme (*CYP17A1*). Further studies will investigate the regulation of miR-320a levels and its effects upon steroid profiles.

Nothing to Disclose: LAD, SW, SMM, JMC, ED

Pub # P2-577

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Steroidogenesis & Its Disorders (1:30 PM-3:30 PM)

Title Lack of Renal 11 β -Hydroxysteroid Dehydrogenase Type 2 at Birth in Humans and Mice: A Targeted Temporal Window for Glucocorticoid Action?

Author String L Martinerie, P Boileau, G Meduri, A-L Delezoide, E Pussard, M Lombes
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Body Glucocorticoid hormones play a critical role in promoting maturation of fetal organs, essential for neonatal adaptation to extrauterine life. On the other hand, excessive glucocorticoid exposure in utero has numerous harmful effects, like reducing fetal growth and increasing susceptibility to the development of hypertension in adulthood. Transfer of maternal glucocorticoids to the fetus is mainly controlled by a placental [ldquo]barrier [rdquo]: the enzyme 11beta-hydroxysteroid dehydrogenase type 2 (11 β HSD2), which metabolizes active glucocorticoids into inactive 11-keto compounds. This enzyme is also expressed in other organs, as the kidney, where it prevents illicit occupation of the mineralocorticoid receptor by glucocorticoids. To investigate the role of renal 11 β HSD2 in the regulation of glucocorticoid metabolism and action in the neonatal period, we evaluated renal 11 β HSD2 activity at birth in human and mice. We measured cortisol and cortisone in maternal plasma, umbilical cord blood and in human newborns urine, using HPLC method. 11 β HSD2 activity was indirectly assessed by comparing the cortisol/cortisone ratio between maternal and neonatal plasma (placental activity) and between plasma and urine in human newborns (renal activity). We demonstrated that, at variance with placental 11 β HSD2 activity, renal 11 β HSD2 activity appears very weak at birth and parallels the low fetal plasma cortisol levels measured in human newborns. These results were corroborated by direct measurement of 11 β HSD2 activity in mouse kidneys at different developmental stages, which confirmed the absence of renal enzymatic activity at birth. To further explore the underlying mechanisms we analyzed renal 11 β HSD2 mRNA and protein expression during gestation and in the postnatal period, both in mice and humans, using quantitative RT-PCR and immunohistochemistry. We found low mRNA levels at birth, concomitant with an absence of detectable 11 β HSD2 protein, whereas an increase in renal mRNA levels and a specific immunolabeling in cortical collecting duct cells were observed in the postnatal period. Altogether, our results demonstrate a weak or absence of neonatal renal 11 β HSD2 activity which is conserved both in human and mice. This temporal and tissue-specific downregulation of 11 β HSD2 activity may represent a physiological window for glucocorticoid action, necessary for optimal fetal and neonatal development but yet could constitute a breach favoring deleterious effects of glucocorticoid excess.

Sources of Research Support: Grants form Inserm, Université Paris-Sud, APHP.

Nothing to Disclose: LM, PB, GM, A-LD, EP, ML

Pub # P2-578

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Steroidogenesis & Its Disorders (1:30 PM-3:30 PM)

Title A Novel Transgenic Mouse Model for Studying the Human Aromatase Gene Regulation *In Vivo*

Author String L Strauss, E Lauren, P Rantakari, A Salminen, K Sjogren, P Pakarinen, P Kujala, C Ohlsson, S Makela, M Poutanen
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Body There are major differences in the promoter region and regulation of the human and rodent aromatase (CYP19A1) genes. Human aromatase is widely expressed in the extragonadal tissues, while the mouse and rat genes are almost exclusively expressed in the gonads and brain. Thus, regarding extragonadal aromatization, the results obtained from rodent models do not translate to human. To generate a model for studying the human aromatase gene regulation in vivo, we have generated several transgenic mouse lines with a construct composing of a 100-kbp-long 5'-region of the human CYP19A1 gene attached to a luciferase reporter gene. The luciferase activity in the tissues was measured in vivo using a CCD camera or ex vivo using a luminometer. For studying the hormonal regulation of the reporter gene, we analyzed the reporter gene expression in prepubertal and adult mice, and in gonadectomized mice treated with testosterone. Immunohistochemical and qRT-PCR analyses were also performed to study the aromatase expression in a set of human tissue samples.
The data revealed that the 100-kbp-long 5'-region of the human CYP19A1 promoter was functional in mouse in vivo. As predicted on the basis of human data, very high reporter gene activity was detected in the gonads and placenta and high/medium expression in, e.g., brain, bone, white adipose tissue, epididymis, penile skin, prostate, uterus and mammary gland, while no expression was observed in liver. Surprisingly, very high luciferase activity was also detected in the seminal vesicles, suggesting a role for seminal vesicles as a source of estrogen in men. Using qRT-PCR and immunohistochemistry, we confirmed that aromatase is expressed in the human seminal vesicles. Moreover, we showed that the expression of the reporter gene in the mouse seminal vesicles was regulated by androgens. In conclusion, we have generated a mouse model for studying the tissue-specific regulation of the human aromatase gene in vivo, and identified the seminal vesicles as previously unknown site for estrogen production in men.

Sources of Research Support: Academy of Finland, Sigrid Juselius Foundation.

Nothing to Disclose: LS, EL, PR, AS, KS, PP, PK, CO, SM, MP

Pub # P2-579

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Steroidogenesis & Its Disorders (1:30 PM-3:30 PM)

Title Site-Specific Overexpression of Urocortin 2 Results in Modulation of Steroidogenesis *In Vivo*

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Body Urocortin 2 (Ucn2) is a recently identified neuropeptide of the CRF-family, involved in homeostatic mechanisms for stress response and control of anxiety. To further elucidate Ucn2 effects on steroidogenesis, we developed a mouse model with Ucn2 overexpression in the adrenals, gonads and parts of the hypothalamus, by crossbreeding SF1-Cre^{+/-} mice with R26^{stop} UCN2/stop UCN2 animals. Following genotypes were obtained: SF1-Cre^{-/-} R26^{+/-stop} UCN2 (controls) and SF1-Cre^{+/-} R26^{+/-stop} UCN2 (Ucn2OE). Adrenal glands, ovaries, testes and trunk blood of two month old animals were collected. Plasma concentration of corticosterone and Ucn2 were measured with commercially available immunoassays. Expression levels of Ucn2 and steroidogenic genes were quantified by Real-Time PCR analysis. A 3.5-fold elevated plasma Ucn2 (P<0.001) and a 20-fold increased Ucn2 expression in adrenals (P<0.001) of male and female Ucn2OE could be documented. A lower expression of StAR (P<0.05), cyp11a1 (P<0.001) and cyp11b1 (P<0.05) could be demonstrated in adrenals of female Ucn2OE, together with reduced plasma corticosterone values (P=0.05). In contrast, 6-fold higher Ucn2 expression in ovaries was associated with significant increase of StAR (P<0.05) and cyp11a1 (P<0.05). On the contrary, steroidogenic enzymes' expression in male adrenals and plasma corticosterone levels did not differ from controls (StAR P=0.29, cyp11a1 P=0.70, cyp11b1 P=0.99, corticosterone P=0.47). Surprisingly, Ucn2 expression in testes was comparable to controls and no effects on steroidogenesis could be documented. These data demonstrate that Ucn2 overexpression in the female mouse results in downregulation of steroidogenesis in the adrenal, suggesting a stress coping behaviour and increased steroidogenesis in the ovary, an observation not confirmed in male gonads. The gender specific discrepancies will be further investigated.

Nothing to Disclose: AS, AH, KM-P, JD, FB

Pub # P2-580

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Steroidogenesis & Its Disorders (1:30 PM-3:30 PM)

Title p53 Is a Negative Regulator of Aromatase in Human Breast Adipose Stromal Cells

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Body

The majority of postmenopausal breast cancers are oestrogen receptor positive and rely on oestrogens produced within the breast for increased growth. The aromatase enzyme is responsible for the conversion of androgens to oestrogens and its expression is upregulated in response to tumor-derived factors in breast adipose adjacent to a tumor. P53 is a known tumor suppressor and its loss of function in cancer is well characterized.

We have identified three putative p53 response elements in aromatase promoter PII. Therefore, this study aimed to examine the role of p53 in regulating aromatase expression in human breast adipose stromal cells (hASCs) in the context of postmenopausal breast cancer. Primary hASCs were obtained from breast reduction surgery and real-time PCR was performed to examine p53, aromatase and 18S (housekeeping gene) transcript expression. Luciferase assays were performed to assess PII activity in COS-7 cells. Treatments were PGE2 (1 [mu]M) or FSK/PMA (25 [mu]M forskolin/4 nM phorbol ester; to mimic tumor-derived factor PGE2) and/or 10 [mu]M RITA (reactivation of p53 and induction of tumor cell apoptosis; a p53 stabilizer). Results demonstrate that RITA significantly decreased the PGE2-dependent induction of aromatase transcript expression (P[le]0.05) and the FSK/PMA-dependent activation of aromatase PII (P[le]0.01). Moreover, FSK/PMA significantly decreased p53 transcript expression in hASCs (P[le]0.01). Chromatin immunoprecipitation assays demonstrated binding of p53 to aromatase PII under resting conditions in hASCs, and this interaction was decreased in the presence of FSK/PMA. Consistent with this, luciferase assays using series of deletion constructs revealed that p53 effects on aromatase PII were due to regions encompassing the proximal putative p53 binding site. This study therefore identifies p53 as a negative regulator of aromatase expression, and builds on our previous findings examining regulators of aromatase expression in the context of metabolism and cancer.

Sources of Research Support: NHMRC CDA to KAB; NHMRC project grant 1005735 to KAB; VBCRC to ERS.

Nothing to Disclose: KAB, LW, IC-O, MD, ERS

Pub # P2-581

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Steroidogenesis & Its Disorders (1:30 PM-3:30 PM)

Title Structure-Activity Relationship Studies of *N*-Phenylanthranilic Acid-Based AKR1C3 Inhibitors

Author String AO Adeniji, BM Twenter, MC Byrns, Y Jin, JD Winkler, TM Penning
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Body Aldo-keto reductase 1C3 (AKR1C3) also known as type 5,17 β -hydroxysteroid dehydrogenase (17 β -HSD), has been implicated in the development of castrate resistant prostate cancer (CRPC). CRPC is associated with increased androgen receptor (AR) signaling brought about by elevated intratumoral androgen biosynthesis among other factors. AKR1C3 catalyzes the formation of testosterone and the proliferative prostaglandins F_{2c}. AKR1C3 inhibition will deprive the tumor of needed proliferative factors and is an appealing molecular target for management of CRPC.

Small molecule inhibitors of AKR1C3 with little or no effect on the related isoforms, AKR1C1 and AKR1C2 are desirable in the context of CRPC as the latter enzymes are involved in the inactivation of 5 α -dihydrotestosterone. NSAIDs and *N*-phenylanthranilates (*N*-PA) in particular are known to be non-selective AKR1C3 inhibitors at concentrations required for cyclooxygenase inhibition. Using the *prototype N*-PA, flufenamic acid, as a lead compound, analogs representing modifications on the two aromatic rings were evaluated for AKR1C3 inhibitory activity.

Structure activity relationship studies of these compounds showed that the *meta*-carboxylic acid group was essential for AKR1C3 selectivity. Also, additional substitution on the A-ring generally led to similar or lower AKR1C3 inhibitory potency with no major effect on the selectivity. The 4[prime] substituted 3-(phenylamino) benzoic acids were found to be the most potent and selective AKR1C3 inhibitors. These analogs displayed nanomolar affinity and significant selectivity for AKR1C3. Their activity correlated with the interdependent physicochemical parameters, electronic effects of the B-ring substituents and pK_a of the secondary amine and carboxylic acid. X-ray crystal structure of a promising analog, 3-((4-(trifluoromethyl) phenyl) amino) benzoic acid with AKR1C3 has also been acquired. **(Crystal Structure of human type 5 17 β -hydroxysteroid dehydrogenase complex (AKR1C3) in complex with 3-((4-(trifluoromethyl) phenyl) amino) benzoic acid- Abstract for ENDO Society meeting 2011)**

These compounds blocked the production of testosterone in LNCaP-AKR1C3 transfected cells and are promising leads for drug development in CRPC and other malignancies with pathologic AKR1C3 activity. They can also find application in elucidating the role of AKR1C3 and AKR1C enzymes in general in biological systems.

Sources of Research Support: In part by 1R01-CA90744 and a Challenge Grant from the Prostate Cancer Foundation, to TMP.

Nothing to Disclose: AOA, BMT, MCB, YJ, JDW, TMP

Pub # P2-582

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Steroidogenesis & Its Disorders (1:30 PM-3:30 PM)

Title Structural Basis of Differences in Activities and Interaction of Steroidogenic P450s Caused by Mutations in Human NADPH P450 Oxidoreductase

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Body Mutations in NADPH P450 oxidoreductase (POR, CPR, CYPOR) are associated with a range of human diseases (1). Since the initial observation of mutations in POR affecting activities of CYP17A1 and causing disruption of steroid metabolism, more than 50 variations in POR gene in humans have been identified (2). Most of the POR variants have been tested to support the 17 α -hydroxylase and 17,20 lyase activities of CYP17A1 but recently activities of some other steroidogenic P450s (CYP19A1 and CYP21A2) as well drug metabolizing P450s (CYP3A4 and CYP2D6) and other partner proteins of POR like cyt b5 and heme oxygenase have been reported by us and other laboratories (3-7). Although some general conclusions can be drawn about POR mutations directly affecting cofactor (FMN, FAD and NADPH) binding, there are differences in effects of POR variants on redox partners. We have sought to examine the structural basis of changes caused by POR variants by making structurally flexible models of POR and docking of P450s as well as correlation of enzymatic and protein-protein interaction data using specific antibodies. Computational modeling using a flexible structural model of POR allowed us to identify POR-P450 contacts and identify potential interacting amino-acid pairs located on POR and P450s to examine the differences in interaction patterns and catalytic activities that are sometimes affected for one particular P450 but not others. Domain motion in POR was also studied and mutations that affect the flexibility and inter-domain interaction in POR were also examined. In POR amino acids D116, E119, E145, D147 and Y181 were found to be actively involved in interaction with partner proteins. For CYP17A1 we identified Q122, R126, F135, K136 and K141 for CYP19A1 K142, R145, F147, K150, and R159, and for CYP21A2 K118, K122, R125, R133 and M136 were found to be important for interaction with POR. A detailed analysis of different POR variants causing specific changes in interaction with redox partners and effects on activities will be presented.

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- (2) Flück CE and Pandey AV. Endo Dev 2011; 20:63
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Sources of Research Support: Swiss National Science Foundation 3100A0-113719, Swiss Society for Endocrinology and Diabetology, Bern University Research Foundation.

Nothing to Disclose: AVP

Pub # P2-583

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Steroidogenesis & Its Disorders (1:30 PM-3:30 PM)

Title Up-Regulation of mRNA Levels of 11 β -Hydroxysteroid Dehydrogenase Type 1 in the Liver of Morbidly Obese Patients with Metabolic Syndrome

Author String E Torrecilla-Garcia, G Fernandez-Vazquez, D Vicent-Lopez, F Sanchez-Franco, L Cabrerizo-Garcia, A Sanchez-Pernaute, AJ Torres-Garcia, MA Rubio-Herrera
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Body Local hepatic and adipose tissue cortisol regeneration from cortisone via 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1) is dysregulated in obesity and the metabolic syndrome (MS). In vivo studies showed that hepatic 11 β -HSD1 activity is down-regulated in obesity. This has been interpreted as a compensation to prevent metabolic disorders. However, hepatic 11 β -HSD1 down-regulation fails in obese diabetic patients. Therefore the lack of this compensation can lead to metabolic abnormalities in obese subjects. To better characterize the tissue-specific cortisol regeneration in obesity, we have investigated the mRNA expression of genes related to local cortisol production/action, i.e., 11 β -HSD1, hexose-6-phosphate dehydrogenase (H6PD) which provides NADPH for 11 β -HSD1 reductase activity, and glucocorticoid receptor (GR) in the liver, visceral (VAT) and subcutaneous (SAT) adipose tissues from morbidly obese patients with and without MS. Fifty morbidly obese patients, 14 males and 36 women, age 16-70 and body mass index 35-78, were classified as having (MS+, n= 20) or not (MS-, n= 30) MS according to NCEP-ATP III definition. mRNA levels were measured by real-time PCR in tissues obtained during bariatric surgery. Hepatic 11 β -HSD1 mRNA levels were higher in obese patients with MS (MS+: 20.97 \pm 9.3 vs MS-: 7.67 \pm 2.22; P = 0.002) and positively correlated with the number of clinical characteristics that define the MS (P = 0.003). Hepatic 11 β -HSD1 mRNA levels negatively correlated with serum HDLc (P = 0.026) but not statistically significant associations were observed with other clinical or biochemical variables. H6PD mRNA levels were also higher in the liver of patients with MS (MS+: 6.19 \pm 2.64 vs MS-: 3.58 \pm 0.86; P = 0.04) and correlated positively (P = 0.04) with the number of parameters of the MS. Hepatic GR mRNA levels were also higher in patients with MS (MS+: 8.34 \pm 3.22 vs MS-: 4.45 \pm 0.78; P = 0.03) and positively correlated with the number of clinical parameters of the MS (P = 0.001). mRNA levels of the three genes positively correlated among them. None of the genes are differently expressed in VAT or SAT, when obese patients with and without MS were compared. Liver-specific up-regulation of genes involved in local cortisol regeneration and action supports the concept that a relative intrahepatocytic hypercortisolism may contribute to development of MS in morbidly obese patients.

Sources of Research Support: Fundación Mutua Madrileña Research Project P-007; Award Research 2009 from SENDIMAD (Endocrine Society of Madrid).

Nothing to Disclose: ET-G, GF-V, DV-L, FS-F, LC-G, AS-P, AJT-G, MAR-H

Pub # P2-584

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Steroidogenesis & Its Disorders (1:30 PM-3:30 PM)

Title Gender-Specific Restraint of ACTH Secretion in a Continuous and Pulsatile Cortisol Infusion Model

Author String P Aoun, JR Wigham, SM Weist, KM Daniel, JM Miles, VD Johannes
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Body **HYPOTHESIS:** Continuous and pulsatile cortisol infusions exert unequal negative feedback on ACTH secretion, which is further modulated by gender. **METHODS:** Preliminary aggregate data on 25 (15M/10F) healthy adults, ages 45-76, pretreated with leuprolide and either placebo or sex-steroid addback. Subjects were assigned randomly to 4 overnight visits in a within-subject, placebo-controlled crossover design. Experimental interventions included: 1) oral placebo and a 14-h saline infusion (22:00-12:00), 2) saline infusion during reversible inhibition of adrenal cortisol synthesis with ketoconazole (KTCZ), 3) KTCZ + 14-h continuous cortisol infusion (7 mg/m²/14h), and 4) KTCZ + 10-min cortisol pulses every 90 min (total 7 mg/m²/14h). Blood was sampled every 10 min for a total of 8 h (04:00-12:00) for measurement of cortisol and ACTH. Oral hydrocortisone was given at the end of each session. The primary endpoints were mean, peak, and nadir ACTH concentrations and approximate entropy (a sensitive measure of ensemble feedback control). Results were further separated by gender. **RESULTS:** The paradigm achieved statistically comparable mean cortisol concentrations during both continuous and pulsatile infusions compared with double placebo (10 mcg/dL ± 0.3). Cortisol peaks were lower (p<0.001) in the continuous (13 mcg/dL ± 0.43) and pulsatile (14 mcg/dL ± 0.5) groups compared with placebo (18 mcg/dL ± 0.52). Cortisol nadirs were higher in the continuous (8.1 mcg/dL ± 0.46; p<0.01) but not pulsatile group relative to placebo. Both infusions normalized mean, peak, and nadir ACTH concentrations relative to the double placebo (19 ± 1.3; 38 ± 3; and 10 ± 0.77 ng/L, respectively). Cross-approximate entropy of cortisol feedback on ACTH showed greater irregularity during both continuous (1.18 ± 0.055) and pulsatile (1.16 ± 0.03) infusions compared with placebo (0.94 ± 0.038; p<0.01), but did not differ between the two cortisol infusion groups. Stratification of the data by gender in a 3-way ANCOVA model showed a statistically higher mean ACTH concentration in men compared with women (p=0.007) which was not explained by a difference in cortisol concentrations between the two groups. **CONCLUSION:** Both continuous and pulsatile cortisol infusions restrain ACTH secretion albeit with incomplete normalization of irregularity and distinct relative gender effects. We postulate that normalized regularity requires adequate peak cortisol concentrations.

Sources of Research Support: DK73148.

Nothing to Disclose: PA, JRW, SMW, KMD, JMM, VDJ

Pub # P2-585

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Steroidogenesis & Its Disorders (1:30 PM-3:30 PM)

Title Novel Bacterial Expression of Human P450 Oxidoreductase (POR) and Kinetics of Electron Transfer from NADPH: Comparison of the Wild-Type Enzyme and Two Mutants

Author String Y Jin, D Sandee, WL Miller
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Body POR is a 2-flavin protein that transfers electrons from NADPH via its FAD and FMN moieties to all microsomal cytochrome P450 enzymes, including steroidogenic P450c17, P450c21 and P450aro and all hepatic drug-metabolizing P450 enzymes. POR mutants cause phenotypes ranging from genital anomalies with the Antley-Bixler skeletal malformation syndrome (ABS) to phenotypically normal persons with infertility. Different POR variants exert differential effects on P450s in multiple assays in vitro. For example, Q153R causes ABS and impaired the 17-hydroxylase and 17,20 lyase activities of P450c17 in vitro, but showed gain-of-function with hepatic CYP1A2, CYP2C19, CYP2D6 and with some activities of CYP3A4, whereas A287P, found in 40% of Caucasian ABS patients, dramatically reduced all of these activities. Novel vectors expressing N-27 human wild-type (WT), Q153R and A287P POR with C-terminal Gly3His6 tails were built to facilitate rapid high-purity preparation; POR expressed in E.coli was purified to apparent homogeneity by Ni-NTA chromatography, and had the expected adsorption maxima and the same specific activity as conventionally prepared POR to support cytochrome c reduction and the 17-hydroxylase and 17,20 lyase activities of P450c17. We measured the transfer of electrons from NADPH to POR by anaerobic stopped-flow spectrometry, monitoring absorbance changes at 450 nm for electron transfer from NADPH to FAD and at 590 nm for the interflavin transfer between the FAD and the FMN components of POR. Q153R behaved similarly to WT POR, showing slightly faster (1.3 fold) NADPH to FAD transfer (87.8 s^{-1} Q153R vs 68.0 s^{-1} WT) and the same FAD to FMN transfer (83.2 s^{-1} Q153R vs. 82.4 s^{-1} WT). By contrast, the fast phase of the 450 nm absorbance decay was not observed with A287P, suggesting the electron transfer from NADPH to FAD was significantly slower ($1-3 \text{ s}^{-1}$ A287P) than with WT. The transient kinetic data indicated that the ability of POR to accept electrons from NADPH and to transfer electron from FAD to FMN were not affected by the Q153R mutation, but dramatically reduced by the A287P mutation. Thus the differential effects of Q153R on various P450s were due to difference in FMN to P450 electron transfer with different P450 partner, whereas the reduced rates of P450s with A287P may be due to the impaired reaction of A287P with NADPH.

Sources of Research Support: In part by DK47015 and GM073020.

Nothing to Disclose: YJ, DS, WLM

Pub # P2-586

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Steroidogenesis & Its Disorders (1:30 PM-3:30 PM)

Title Does AMP-Activated Protein Kinase [ldquo]Fuel[rdquo] Androgen Biosynthesis in Human Adrenal NCI-H295R Cells?

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Body AMP-activated protein kinase (AMPK) is a sensor of nutritional stress and maintains the balance between the production and consumption of ATP across eukaryotes. In mammals, AMPK plays a crucial role in fuel metabolism like gluconeogenesis and fatty acid oxidation in the liver and glucose uptake and fatty acid oxidation in muscles. Therefore, AMPK attracted recently attention as a potential drug target in the treatment of the metabolic syndrome. AMPK has been reported to be regulated by metformin, a drug widely used in the treatment of diabetes and the polycystic ovary syndrome (PCOS). As PCOS also affects steroidogenesis of the adrenals and ovaries, we thought of a role of AMPK in the regulation of androgen biosynthesis. Therefore, we investigated the effects of AMPK signaling on androgen biosynthesis in human adrenal NCI-H295R cells. RT-PCR experiments demonstrated that NCI cells and human adrenal tissue express all three subunits of AMPK. Overexpression of the catalytic α subunits of AMPK did not change the steroid profile, as assessed by substrate labeling and thin layer chromatography. Unfortunately, transient as well as stable transfection with shRNA constructs failed to silence AMPK α because it consistently killed the NCI-R cells suggesting that AMPK is crucial for cell survival and metabolism. Effect of AMPK signaling was studied on the CYP17A1 and 3 β -hydroxysteroid dehydrogenase type 2 (3 β HSDII) genes, which are key players of androgen biosynthesis. Compound C, an inhibitor of AMPK, decreased 17 α -hydroxylase and 17,20-lyase activity of CYP17A1. In contrast, the activator of AMPK, 5-aminoimidazole-4-carboxamide (AICAR), increased phosphorylation of AMPK α and enhanced 17,20-lyase activity. The activity of 3 β HSDII was neither altered by AICAR nor compound C. Enzyme kinetic assays revealed that compound C directly inhibited CYP17A1 activities. By contrast, Western blot showed that compound C increased the amount of CYP17 protein in cells, similar to a protease inhibitor. In summary, the AMPK signaling pathway seems to regulate androgen production in NCI cells. AICAR, stimulator of AMPK, enhances CYP17A1-17,20-lyase activity. The AMPK inhibitor compound C directly inhibits CYP17 activities and may even act as a protease inhibitor; therefore, it can not be used for specific AMPK signaling studies in steroid cells.

Nothing to Disclose: AH, PK, GH, PEM, CEF

Pub # P2-587

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Steroidogenesis & Its Disorders (1:30 PM-3:30 PM)

Title Partial Mixed Oxidase Disease Brought by P450 Oxidoreductase Mutation P399_G401del: Characterization of Phenotype-Genotype and Structure-Function

Author String CE Fluck, D Mallet, D Samara-Boustani, J Leger, M Polak, Y Morel, AV Pandey
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Body P450 oxidoreductase (POR) is the electron donor for all microsomal P450s including steroidogenic enzymes CYP17A1, CYP19A1 and CYP21A2. POR deficiency may manifest with a severe Antley-Bixler syndrome (ABS)-like phenotype with skeletal malformations and genital anomalies or only cause minor abnormalities in steroid hormone production, manifesting as polycystic ovary syndrome with fertility issues. Many POR variants have differences in interaction and regulation of the activities of various P450s, and have to be studied individually. We found a novel POR mutation P399_G401del in two Turkish families. Patient 1 was born to consanguineous parents with a 46,XX disorder of sexual development (DSD) manifesting as genital virilization (Prader V) and mild ABS features. Biochemically, she had high baseline progesterone, moderately elevated 17OHProg and low androgens; cortisol did not increase upon ACTH stimulation. Patient 2 also had consanguineous parents and was born with 46,XX DSD with genital ambiguity Prader III. She had severe ABS phenotype. Her hormonal data show grossly elevated Prog, moderately elevated 17OHProg, low androgens at baseline and lack of cortisol response to ACTH stimulation. For functional analysis, recombinant POR proteins were produced in yeast and tested for their ability to support steroid metabolizing enzyme activities. In comparison to wild-type POR, the P399_G401del protein was found to decrease catalytic efficiency of 21-hydroxylation of progesterone by 68%, 17-hydroxylation of progesterone by 76%, 17,20-lyase action on 17OH-pregnenolone by 69%, and aromatization of androstenedione by 85%. Ferricyanide reduction activity of P399_G401del POR was similar to WT and cytochrome c reduction activity was 75% of WT. Protein structure analysis of the three amino acid deletion P399_G401 revealed reduced flexibility of the mutant. Docking studies with various interaction partners using an open conformation of POR showed differences in interaction pattern for P450s that may account for reduced activities. In conclusion, P399_G401del is a novel mutation in POR that provides valuable genotype-phenotype and structure-function correlation for mutations in a different region of POR compared to previous studies that will provide further insights into specificity of different P450s for interactions with POR as well as nature of metabolic disruption caused by more pronounced effect on specific P450s like CYP17A1 and aromatase.

Nothing to Disclose: CEF, DM, DS-B, JL, MP, YM, AVP

Pub # P2-588

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Steroidogenesis & Its Disorders (1:30 PM-3:30 PM)

Title Role of Adiponectin in the Regulation of Steroidogenic Acute Regulatory (StAR) Protein: Implication in Human Adrenal Steroidogenesis

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Body Steroidogenesis is regulated by variety of neuropeptides and adipokines. Adiponectin is an abundantly circulating adipokine, orchestrating its effects through two 7-transmembrane receptors (AdipoR1 and AdipoR2). Earlier studies have reported adipokine mediated steroid production. A key rate-limiting step in steroidogenesis is cholesterol transportation across the mitochondrial membrane by steroidogenic acute regulatory protein (StAR). Several signalling pathways regulate StAR expression. The actions of adiponectin and its role in human adrenocortical steroid biosynthesis are not fully understood. The aim of this study was to investigate the effects of adiponectin on StAR protein expression, steroidogenic genes, cortisol production and to dissect the signalling cascades involved in activation of StAR expression. Using qRT-PCR, Western blot analysis and ELISA, we have demonstrated that stimulation of human adrenocortical H295R cells with adiponectin results in increased cortisol secretion. This effect is accompanied by increased expression of key steroidogenic pathway genes including StAR protein expression *via* ERK1/2 and AMPK-dependent pathways. This has implications for our understanding of adiponectin receptor activation and peripheral steroidogenesis. Finally, our study aims to emphasise the key role of adipokines in the integration of metabolic activity and energy balance partly via the regulation of human adrenal steroid production.

Nothing to Disclose: MR, ACC, HL, JC, HSR

Pub # P2-589

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Steroidogenesis & Its Disorders (1:30 PM-3:30 PM)

Title Interaction of Growth Hormone and Bone Morphogenetic Protein System in Steroidogenesis by Rat Granulosa Cells

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Body Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan

GH induces preantral follicle growth and differentiation via GHR in oocytes and follicular somatic cells. GH stimulates development of small antral follicles to secondary stages with oocyte maturation. However, GH effects on ovarian steroidogenesis and the detailed mechanism have yet to be clarified. Here we investigated GH actions on steroidogenesis in early antral follicles from immature female rats by focusing on ovarian BMP system. BMPs play a key role in normal folliculogenesis in mammals, in which BMP ligands commonly suppress FSH-induced progesterone production. It was revealed that GH suppressed FSH-induced estradiol production with reducing aromatase expression, while GH increased FSH-induced progesterone level with induction of StAR, P450scc and 3 β HSD expression. However, GH did not affect steroids production induced by cAMP compounds. Since GH effects on FSH-induced steroid production were not accompanied by changes in cAMP synthesis by granulosa cells, GH actions on ovarian steroidogenesis were not directly mediated via cAMP-PKA. In addition, GH treatment increased the expression levels of IGF-1 and IGF-1R in granulosa cells. It was thus suggested that endogenous IGF-1 is likely to be involved in the GH regulation of steroidogenesis. It was of note that GH and IGF-1 had synergistic effects on FSH-induced activation of MAPKs including ERK1/2 and p38 pathways, while and GH-induced STAT phosphorylation was not affected in the presence of FSH. IGF-1 treatment showed increasing effects on estradiol and progesterone production induced by FSH and inhibition of IGF-1R signaling suppressed both FSH-induced estrogen and progesterone levels. Moreover, neutralization of endogenous IGF-1 by anti-IGF-1 antibody restored the GH stimulation of FSH-induced progesterone production, suggesting that endogenous IGF-1 is functionally involved in GH effects on progesterone induction. Of note, BMP treatment suppressed GHR and IGF-1R expression in granulosa cells. On the other hand, GH and IGF-1 suppressed BMP-Smad1/5/8 signaling through downregulating the expression of BMP type-2 receptors in granulosa cells. Thus, GH acts to modulate estrogen and progesterone production differentially through the endogenous IGF-1 activity in granulosa cells, in which the GH-IGF-1 interaction leads to antagonizing BMP signaling in granulosa cells. The mutual balance between IGF-1 and BMPs may be a key for regulating GH-induced steroidogenesis in the growing follicles.

Nothing to Disclose: EN, FO, KI, TM, NT, MT, MT, JS, TO, HM

Pub # P2-590

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Steroidogenesis & Its Disorders (1:30 PM-3:30 PM)

Title Effect of SOM230 on Human and Rat Adrenal Secretion *In Vitro*

Author String F Pecori Giraldi, L Pagliardini, MF Cassarino, F Martucci, L Castelli, E Montanari, F Cavagnini
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Body SOM230, a recently developed somatostatin analogue, has been tested in patients with Cushing's disease with promising results. Experimental evidence indicates a pituitary site of action but no data is yet available on any direct adrenal effect on cortisol secretion. This aspect of the analogue's action appears worth investigating as some patients with Cushing's syndrome have been known to experience adrenal insufficiency soon after initiation of SOM230 treatment. Aim of the current study was to evaluate whether SOM230 modulates corticosteroid secretion by normal adrenals in vitro. Methods: Primary cultures from 6 normal human adrenal and 6 rat adrenals were incubated with 10-100 nM SOM230 with and without 10 nM ACTH. Cortisol/corticosterone levels in medium were measured after 4 and 24 hours. Results: 10 nM SOM230 significantly increased corticosteroid levels after 24h incubation in both human ($131.4 \pm 1.55\%$ of baseline, $p < 0.05$) and rat ($138.1 \pm 15.99\%$, $p < 0.05$) adrenals; lesser effects were observed with 100 nM SOM ($120.4 \pm 9.33\%$ $p < 0.05$; $117.3 \pm 15.49\%$ N.S., for human and rat adrenals, respectively). The corticosteroid secretory response to ACTH was unaffected by SOM230 co-incubation (at 24h human adrenals: cortisol $105.6 \pm 7.16\%$ and $108.4 \pm 6.99\%$ of ACTH-stimulated wells for 10 nM and 100 nM SOM230, NS, respectively; rat adrenals: corticosterone 98.3 ± 12.11 and $91.8 \pm 12.55\%$ of ACTH-stimulated wells for 10 nM and 100 nM SOM230, respectively, NS.) Conclusions: SOM230 exerted an unexpected, moderate stimulatory effect on adrenal corticosteroid secretion in vitro. This argues against an additional, direct adrenal inhibitory effect of SOM230 in patients with Cushing's syndrome.

Nothing to Disclose: FPG, LP, MFC, FM, LC, EM, FC

Pub # P2-591

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Steroidogenesis & Its Disorders (1:30 PM-3:30 PM)

Title Prolonged *In Vitro* Exposure of Normal Human Fasciculata Cells to Non-Esterified Fatty Acids Potentiates Their Androgen Secretion, Which Is Normalized by PPAR γ Agonist

Author String CG Baptiste, M-C Battista, S Bellanger, N Gallo-Payet, J-P Baillargeon
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Body **BACKGROUND:** Androgen biosynthesis is mainly performed by ovarian and adrenal glands. In ovaries, theca and interstitial cells are responsible for the production of androgens; while in the adrenal gland, androgens are secreted by reticularis and fasciculata cells. Polycystic ovary syndrome (PCOS) affects 6-10% of women in their reproductive age and is mainly characterized by hyperandrogenism. Also, PCOS women often display resistance to insulin and higher levels of circulating non-esterified fatty acids (NEFA). Our hypothesis is that prolonged *in vitro* exposure of normal human fasciculata cells to NEFA increases androgen production. We also propose that treatment with a PPAR γ agonist, which promotes NEFA intracellular oxidation, decreases lipotoxicity and restores normal androgen biosynthesis.

METHOD: Primary cultures of human fasciculata cells isolated from organ donors provided by Quebec Transplant were treated for 3 days with palmitate (NEFA; 100 [μ M], 2x/day) or vehicle (Control) in the absence or presence of ACTH (physiologic stimulator of fasciculata cells; 10nM, 2x/day) or rosiglitazone (ROSI; PPAR γ agonist; 1 [μ M], 1x/day). Secretions of DHEA, androstenedione (A₄), and 8-isoprostane (a marker for cellular oxidative stress induced by NEFA) were measured in the culture medium by ELISA. Results were adjusted for the number of cells.

RESULTS: In basal conditions, fasciculata cells exposed to palmitate showed no change in secretion of DHEA (n=3), A₄ (n=3) and 8-isoprostane (n=1) compared to controls. ACTH stimulation increased androgenic and 8-isoprostane secretions and these responses were potentiated with co-treatment with NEFA up to 3-fold for the androgens and up to 8-fold for 8-isoprostane. ROSI alone (n=1) had no impact on studied parameters, but in cells treated both with ACTH and NEFA, ROSI seemed to normalize androgens (n=1) and 8-isoprostane (n=1) secretions to the levels achieved with ACTH alone (n=3).

CONCLUSION: These preliminary results suggest that ACTH-stimulated human fasciculata cells exposed *in vitro* to NEFA generate oxidative stress and increase their androgen secretion, as compared to non-exposed cells. NEFA-induced oxidative stress and hyperandrogenism seems to be corrected by PPAR γ activation, which is known to improve intracellular metabolism of NEFA. These novel findings support a significant role of intracellular NEFA overflow on androgen secretion in androgen-secreting cells, possibly through the induction of cellular oxidative stress.

Nothing to Disclose: CGB, M-CB, SB, NG-P, J-PB

Pub # P2-592

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Steroidogenesis & Its Disorders (1:30 PM-3:30 PM)

Title Differential Inhibition of 11 β -HSD1 Dehydrogenase by 5 β -Ring-a-Reduced Metabolites of 11-Desoxy-Steroid Hormones

Author String SA Latif, R Ge, DJ Morris
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Body Several publications which utilized human preparations of 11 β -HSD isoforms, did not investigate specificity towards the isoforms of 11 β -HSD. Earlier work from our laboratories, had also examined a variety of adrenocorticosteroids and progesterone metabolites for their inhibitory properties against the dehydrogenase activities of 11 β -HSD1 and 11 β -HSD2; these studies did not include their effects against 11 β -HSD1 reductase. These studies indicated that 11 β -HSD1 dehydrogenase can be inhibited by both 5 α and 5 β - Ring A-reduced metabolites of certain 11-desoxy-steroid hormones. In addition, our earlier studies demonstrated that only derivatives of steroid hormones possessing the 5 α - Ring A-reduced configuration selectively inhibited 11 β -HSD2 dehydrogenase, whereas their 5 β - derivatives were inactive. The present studies were designed to explore the relative potencies of endogenous 11-desoxy, 5 α and 5 β - Ring A- reduced metabolites of desoxycorticosterone (DOC) and progesterone. These endogenous substances both 5 α - and 5 β -Tetrahydro derivatives were tested not only for their inhibitory properties against 11 β -HSD1 dehydrogenase, but also towards 11 β -HSD1 reductase. Freshly prepared rat Leydig whole cells and cell homogenates provided a rich and reliable source of both 11 β -HSD1 dehydrogenase and reductase. Here we report that both 5 α - and 5 β -Tetrahydro derivatives were inhibitors of 11 β -HSD1 dehydrogenase and 11 β -HSD1 reductase; 5 β - being more potent than 5 α -derivatives contrary to 11 β -HSD2 isoform. The IC50's were 1.5 and 7.0 [micro]M for 3 α ,5 β - tetrahydro-DOC and 3 α ,5 β - tetrahydro-progesterone respectively, whereas, the IC50s were 10.1 and 12.0 for 3 α ,5 α - tetrahydro-DOC and 3 α ,5 α - tetrahydro-progesterone, for 11 β -HSD1 dehydrogenase. Similarly, the IC50 for 11 β -HSD1 reductase were 4.9 and 11.0 for 3 α ,5 β - tetrahydro-DOC and 3 α ,5 β - tetrahydro-progesterone, respectively, and 10.1 and 12.0 for 3 α ,5 α - tetrahydro-DOC and 3 α ,5 α -tetrahydro-progesterone; again 5 β -derivatives being more potent than 5 α -derivatives. The physiological role of bidirectional 11 β -HSD1 present in a variety of target tissues in several disease states such as obesity, diabetes and hypertension is slowly emerging. Thus, inhibitors of both 11 β -HSD1 dehydrogenase and 11 β -HSD1 reductase(in particular 3 α ,5 β - tetrahydro-DOC) may be related to several disease processes but also to the design of agents to blunt the regeneration of active glucocorticoids from their 11-dehydro derivatives.

Nothing to Disclose: SAL, RG, DJM

Pub # P2-593

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Steroidogenesis & Its Disorders (1:30 PM-3:30 PM)

Title Endocrine, Metabolic Profile and Phenotype-Genotype Correlation in Patients with Non-Classical Congenital Adrenal Hyperplasia

Author String N Skordis, C Shammas, E Efstathiou, K Kaffe, TC Kyriakides, V Neocleous, LA Phylactou Makarios III, Hospital, Nicosia, Cyprus; The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus; Yale University, New Haven, CT

Body Non Classical Adrenal Hyperplasia (NC-CAH) due to 21-hydroxylase deficiency (21-OHD) is an autosomal recessive disorder characterized by impaired activity in the enzyme required for cortisol and aldosterone production, resulting in highly increased adrenal androgen synthesis. Different degrees of enzyme activity impairment and clinical phenotypes depend on the severity of the underlying defect in the *CYP21A2* gene. The aim of this study was to evaluate the relationship between the genotype and phenotype and the 17-hydroxyprogesterone (17-OHP) levels in a group of 40 Cypriot patients affected by NC-CAH. Each patient had increased plasma levels of 17-OHP at baseline and ACTH-stimulated 17-OHP in the middle/upper part of the published nomogram. By DNA sequencing and MLPA analyses it was revealed that the mutation p.V281I was the most predominant, observed in 70% of the alleles and in 92.5% of the patients. The second most frequent mutation was p.P453S (6.25%), followed by IVS2-13A/C (5%), p.Q318X (5%), p.V304M (3.75%), p.M283V (3.75%), p.P482S (3.75%), p.P30L (1.25%), p.P30L+ IVS2-13A/C (1.25%), p.I172N (1.25%) and a large DNA lesion (1.25%).

The patients were divided in two groups based on the genotype (A: mild/mild, B: mild/severe) and no patient showed two severe mutations. Patients in group B had increased levels of basal 17-OHP (37.6 nmol/l). There was no significant difference between group A and group B in terms of signs of hyperandrogenism, except for the presence of PCOS which was 23.3 % and 33.3 %, respectively.

In conclusion, our cohort of patients show a proportional relationship between the type of mutation and the levels of 17-OHP. Additionally, the fact that several mutations of the *CYP21A2* gene were identified in patients with NC-CAH, with p.V281I being the most dominant, precludes the use of genotyping *CYP21A2* alleles for correct 21-OHD phenotype prediction, genetic counseling and treatment.

Nothing to Disclose: NS, CS, EE, KK, TCK, VN, LAP

Pub # P2-594

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Steroidogenesis & Its Disorders (1:30 PM-3:30 PM)

Title Development of Stable Isotope Dilution Liquid-Chromatography Mass Spectrometry (LC/MS) Methods for the Determination of the Androgen Metabolome

Author String MC Byrns, IA Blair, TM Penning
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Body Metastatic prostate cancer is initially responsive to androgen ablation therapy, but eventually recurs as castrate resistant prostate cancer (CRPC). The success of the CYP17A1 inhibitor abiraterone acetate in the treatment of CRPC indicates that this disease remains hormonally driven. Determination of the androgen metabolome in both tumor and serum samples would elucidate which enzymatic steps contribute to androgen biosynthesis and are most likely to respond to pharmacotherapy for CRPC. Analysis of the androgens in serum samples from patients undergoing treatment for prostate cancer could also provide an early predictor of the success of the treatment regimen and aid in tailoring personalized treatments. To this end, we developed stable isotope dilution LC/MS methods for the determination of androgens in ether extracts from biospecimens. Direct detection of androgens by LC/MS lacks sensitivity, particularly for the 5 α -reduced products, so derivatization of ketosteroids as Girard T oximes and hydroxysteroids as picolinic esters has been achieved. These derivatives are much more readily ionized and introduce reliable mass transitions for every analyte, allowing detection of pg or lower quantities of all of the androgens. The derivatized steroids are separated with reverse phase HPLC and quantified through comparison to deuterated internal standards using electrospray ionization MS detection. Detection of conjugated androgens was achieved following treatment of aqueous fractions with glucuronidase and sulfatase. Using Girard T derivatization for ketoandrogens, we analyzed androgen levels in samples from patients undergoing hormone suppressive therapy for prostate cancer. As expected, treatment with leuprolide significantly reduced levels of testosterone, but had a limited effect on other androgens, while combination therapy with leuprolide and abiraterone acetate drastically reduced the levels of all of the androgens analyzed.

Sources of Research Support: 1R01-CA90744 to TMP.

Nothing to Disclose: MCB, IAB, TMP

Pub # P2-595

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Steroidogenesis & Its Disorders (1:30 PM-3:30 PM)

Title Normal Bovine Ovarian Cells Exposed *In Vitro* to Non-Esterified Fatty Acids Display Hyperandrogenism

Author String CG Baptiste, M-C Battista, J-P Baillargeon
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Body

BACKGROUND: Polycystic ovary syndrome (PCOS) is a common but poorly understood condition. Indeed 6-10% of women of reproductive age are affected by this condition that is mainly characterized by clinical or biochemical hyperandrogenism. PCOS women are also more often resistant to insulin and present higher levels of circulating non-esterified fatty acids (NEFA). Our hypothesis is that prolonged *in vitro* exposure of normal ovarian cells to NEFA induces an increase in their androgen production. We also propose that treatment with a PPAR γ agonist, which promotes NEFA intracellular oxidation, decreases lipotoxicity and thus restores normal androgen biosynthesis.

METHOD: Experiments were carried out with one bovine ovary obtained from slaughterhouse, in duplicates or triplicates. In primary culture, mixed theca-interstitial and granulosa ovarian cells proliferated from explants for seven days in a defined proliferative medium. Cells were then treated for 24 hours with palmitate (AGNE; 100 [micro]M, 2 x/day) or vehicle (Control) in the absence or presence of LH (physiologic steroidogenic stimulator, 100 ng/ml, 1 x/day) or rosiglitazone (ROSI; PPAR γ agonist, 1[micro]M, 1 x/day). Progesterone (PROG; substrate of 17 α -hydroxyprogesterone (17 α -OHPg), 30[micro]M, 1 x/day) was added to all cultures. Secretions of 17 α -OHPg and 8-isoprostane (marker for cellular oxidative stress induced by AGNE) were measured in the culture medium by ELISA and adjusted for the number of cells.

RESULTS: As compared to control condition, palmitate alone tripled 17 α -OHPg secretion, which was stimulated 1.8-fold by LH alone. When exposed to both LH and palmitate, the effect was additive compared to control cells (5-fold increase). Rosiglitazone seemed to reverse this additive effect. 8-Isoprostane formation did not seem to be affected by any of these treatments.

CONCLUSION: These preliminary results suggest that when exposed to NEFA alone or in combination with LH, theca cells increase considerably their androgen production. ROSI seems to counteract NEFA-induced hyperandrogenemia probably by improving NEFA intracellular metabolism. However, cellular oxidative stress does not seem to be implicated in this model.

Nothing to Disclose: CGB, M-CB, J-PB

Pub # P2-596

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Steroidogenesis & Its Disorders (1:30 PM-3:30 PM)

Title Non-Steroidal Anti-Inflammatory Drug Use and Levels of Estrogens and Androgens in Men

Author String M Gates, S Hall, G Chiu, V Kupelian, G Wittert, C Link, A Araujo, J McKinlay
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Body Previous studies suggest that regular use of non-steroidal anti-inflammatory drugs (NSAIDs) may lower estrogen levels in women. One potential mechanism for this association - decreased aromatase activity due to cyclooxygenase inhibition - would also be expected to influence hormone levels and possibly the risk of hormone-sensitive cancers in men; however, no prior studies have assessed NSAID/hormone associations in men. We examined the association between use of prescription (Rx) and over-the-counter (OTC) NSAIDs and levels of estrogens, androgens, and the testosterone:estradiol ratio among men in the Boston Area Community Health Survey. At baseline (2002-05) participants provided a blood sample as close to waking as possible and interviewers assessed use of analgesics during the preceding 4 weeks by direct observation of medication labels and participant self-report. Testosterone and estradiol were measured by competitive electrochemiluminescence immunoassay and liquid chromatography-tandem mass spectrometry, respectively. Our analysis included 1,774 men after excluding participants with missing or outlying hormone values (n=29) and individuals with medical conditions or current/recent use of medications that may influence hormone levels (n=96). We calculated adjusted geometric mean hormone levels for each category of NSAID use and the percent difference in levels for users vs. non-users. There was no significant association between Rx or OTC NSAID use and any hormone examined after adjustment for age, body mass index, race/ethnicity, blood draw characteristics, self-reported health status, alcohol use, marital status, and several comorbidities and medications of interest. For example, geometric mean testosterone levels were 400 ng/dL in non-users, 377 ng/dL in Rx NSAID users, and 410 ng/dL in users of OTC NSAIDs only, while the corresponding levels for estradiol were 21.8, 19.1, and 21.8 pg/mL. These results do not support an association between NSAID use and estrogen or androgen levels in men.

Sources of Research Support: Award numbers R21 DK082652 and U01 DK56842 from the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), National Institutes of Health (NIH). The content is solely the responsibility of the authors and does not necessarily represent the official views of NIDDK or NIH.

Nothing to Disclose: MG, SH, GC, VK, GW, CL, AA, JM

Pub # P2-597

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Steroidogenesis & Its Disorders (1:30 PM-3:30 PM)

Title High Allele Frequency of p.Q258X Mutation and Identification of Novel Missplicing Mutation in the *STAR* Gene in Korean Patients with Congenital Lipoid Adrenal Hyperplasia

Author String J-H Choi, J-M Kim, H-Y Jin, B-H Lee, G-H Kim, JH Lee, H-W Yoo
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Body

Purpose: The steroidogenic acute regulatory (StAR) protein plays a crucial role in steroidogenesis, as it promotes the transfer of cholesterol from outer to inner mitochondrial membrane. Mutations in the gene encoding StAR cause congenital lipoid adrenal hyperplasia (CLAH). This study investigated mutation spectrums and functional analysis of a novel mutation of the *STAR* gene in Korean patients with CLAH.

Patients and methods: Eighteen unrelated patients were diagnosed as having CLAH based both on endocrine profiles and mutation analysis. Seven exons of the *STAR* gene and their intronic flanking sequences were amplified by PCR, and directly sequenced. The cDNA of *STAR* gene from gonadal tissue was cloned into the expression vector, pcDNA3.1, followed by site-directed mutagenesis and transient expression in COS-7 cells. The splicing pattern was analyzed by *in vitro* transcription, and each transcript was functionally characterized

Results: Twelve out of 18 patients were genetic males with female external genitalia. The basal ACTH and plasma renin levels were extremely high in all patients. The c.772C>T (p.Q258X) mutant allele was identified in 32 out of 36 alleles (88.9%). The c.653C>T mutation was detected in two out of 36 alleles (5.6%). The c.545G>A (p.R182H) and c.745-6_810del mutations were found in one out of 36 alleles (2.8%). *STAR* gene analysis using genomic DNA from peripheral blood leukocytes of the patient identified a compound heterozygous for c.653C>T and c.745-6_810del. RT-PCR products amplified from RNA of patient's gonadal tissue were detected multiple alternatively spliced mRNAs. *In vitro* expression analysis of an allelic minigene consisting of exons 4 through 7 containing the c.653C>T mutation yielded two transcripts with exon 6, or exon 5 and 6 skipping. The c.745-6_810del mutation leads to full and partial intron retention. The functional ability of both *STAR* mutations to promote pregnenolone production was severely impaired in COS-7 cells when co-transfected with the mutants and F2 plasmid for the expression of P450scc-adrenodoxin reductase-adrenodoxin fusion protein.

Conclusions: The p.Q258X mutation in the *STAR* gene is the most common in Korean patients. Previously reported c.653C>T variant as a missense p.A218V mutation turned out to cause aberrant splicing at mRNA level, leading to a frameshift at amino acid position 217. Also, the c.745-6_810del mutation results in aberrant splicing, causing perturbation of the StAR function.

Nothing to Disclose: J-HC, J-MK, H-YJ, B-HL, G-HK, JHL, H-WY

Pub # P2-598

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Steroidogenesis & Its Disorders (1:30 PM-3:30 PM)

Title Impacts of Non-Esterified Fatty Acid Exposure on Androgen Synthesis in Bovine Adrenal Fasciculata/Reticulata Cells

Author String S Bellanger, M-C Battista, J-P Baillargeon
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Body **Introduction:** Polycystic ovary syndrome (PCOS) is a frequent endocrinopathy, mainly defined by androgen excess of ovary and adrenal glands origin, but also by high levels of non esterified fatty acids (NEFA). Moreover, PCOS women frequently exhibit higher insulin resistance in comparison to healthy women of similar body mass index. Our preliminary results suggest that lipotoxicity could be directly implicated in PCOS hyperandrogenemia. Regarding insulin signaling pathway in PCOS, it was shown that PCOS women derived-ovarian cells display constitutively reduced MEK/ERK activation related to increased CYP17 activity, which is the key enzyme for androgen synthesis. The objective of this work is thus to study the effect of NEFA exposure on DHEA production, p42/p44 phosphorylation and CYP17 expression in fasciculata cells from bovine adrenal glands.

Methodology: Bovine fasciculata cells were seeded and stimulated without or with ACTH (10 nM, 2 times daily), or forskolin (fsk) (10 [micro]M, once daily) for 2 days. In order to study the effect of NEFA, these cells were also exposed or not to palmitate (100 [micro]M, 2 times daily). DHEA production was assessed in cell media by ELISA, and CYP17 proteic expression as well as p42/p44 phosphorylation (ERK1/2 activation) were assessed by western blotting. DHEA levels were standardized to total cellular protein quantity.

Results: Under control condition (without ACTH or fsk stimulation), DHEA is barely detected and no difference was observed under palmitate exposure for both CYP17 expression and p42/p44 phosphorylation. Under stimulated conditions (in presence of ACTH or fsk), DHEA secretion, CYP17 expression and p42/p44 phosphorylation were increased, as expected. Interestingly, palmitate potentiated the effect of ACTH or fsk on DHEA production and decreased p42/p44 phosphorylation, but did not increase CYP17 expression.

Conclusion: In bovine adrenal fasciculata cells, NEFA exposure potentiates ACTH-stimulated androgen biosynthesis and reduces ACTH-stimulated MEK/ERK pathway activation, which reproduces previous observations in ovarian cells derived from PCOS women. Our results suggest that NEFA could therefore play a role in the pathophysiology of PCOS, possibly via impaired activation of the MEK/ERK pathway.

Nothing to Disclose: SB, M-CB, J-PB

Pub # P2-599

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Steroidogenesis & Its Disorders (1:30 PM-3:30 PM)

Title Resistance Tube Training Elicits Similar Testosterone and Growth Hormone Changes as Weight Training and Running

Author String EC Wuorinen, SJ Wuorinen
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Body INTRODUCTION: Exercise stimulates the secretion of testosterone (T) and growth hormone (GH), resulting in an increased energy availability for exercise and protein synthesis. Adults should engage in a resistance training program to maintain muscle mass for quality of life and stave off health problems such as arthritis, diabetes, and hypertension. Recently, an alternative method to traditional weight training (WT), resistance tubing (RT), has become available to the public as an inexpensive, portable form of resistance. What has not been elucidated is if RT stimulates similar levels of T and GH as traditional WT. It was of interest to observe whether WT, RT, or running (RUN) produced a difference in T and GH secretion.
METHODS: Subjects -8 healthy males ages 18-35 yr. Each completed 3 separate trials; control exercise- 30 min of running at 70% of the subjects' est. max heart rate; WT and RT workouts - 3 sets of 10 repetitions (rep at 70% of their 1 rep max of 6 exercises. All trials were done within 2 wks. Two ml of blood were drawn pre- and post-exercise, separation occurred immediately, and plasma was stored in -70[deg]C freezer until all samples were collected. A RIA kit from MP Biomedicals, Solon, OH was used to determine T levels and an EIA kit from ALPCO, Salem, NH for GH.
RESULTS: A t-test of pre- vs. post-workout T and GH levels showed a significant difference for all 3 conditions: RUN and WT ($p < 0.01$); RT ($p < 0.05$). The % change in T level shows the comparative difference between the types of exercise- RUN: 74%, WT: 52%, and RT: 9%. The % change in GH levels - RUN: 574% WT: 334%, and RT: 609%.
DISCUSSION: Of interest in this study was whether RT would have similar T and GH secretion following a workout as WT. The % change of T in the blood increased slightly more for WT than RT; however the increased pre-RT T levels contributed to the smaller change. There was no significant difference in T or GH post-training as similar concentrations occurred in all 3 trials. RUN resulted in larger increases in T as compared to WT and RT, possibly due to the increased amount of muscle mass utilized and on a continuous basis. The hypothesis that RT would elicit the same levels of T and GH as WT was partially supported, as post-workout levels were similar in concentrations, but the % change was different. Future research: examine whether a change in muscle mass and strength would be similar in WT and RT to indicate protein synthesis in the contractile units.

Sources of Research Support: Norwich University Summer Student Research Program - Stefan Wuorinen.

Nothing to Disclose: ECW, SJW

Pub # P2-600

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Steroidogenesis & Its Disorders (1:30 PM-3:30 PM)

Title Successful Fertility in a Patient with Congenital Adrenal Hyperplasia (CAH) and Ambiguous Genitalia: A Case Report

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Body Congenital adrenal hyperplasia (CAH) is an inborn error of metabolism due to the enzyme 21-hydroxylase deficiency (21-OHD) and is inherited in an autosomal recessive manner. Molecular defects in the *CYP21A2* gene are responsible for the manifestation of this disease. In these patients, cortisol production is impaired and 17-OH-progesterone levels are increased. Because of the increased production of androgens, masculinization occurs in genetically female fetuses. Androgen excess is believed to be one of the major factors responsible for poor fertility outcomes in females with congenital adrenal hyperplasia.

The patient is the third sibling in a five children family. Among these children, the eldest female sibling also had simple virilizing CAH. However, she never underwent any medical therapy and though married, never conceived.

The patient in this case report presented at age 29 years with genital ambiguity and progressive enlargement of clitoris, hirsutism, poor breast development and irregular periods. Lately, there had been social pressure to get married. She wanted to marry only if she was assured of her ability to conceive. The hirsutism score in this patient was 10 and serum testosterone level was 2.5 ng/ml. She underwent genital reconstruction and steroid initiation at age 29 years. Within one year of therapy, she conceived twice. These pregnancies were terminated for personal reasons. Again she conceived at the age of 34 years and gave birth to a normal male child.

Nothing to Disclose: EM, BK, AK, ACA

Pub # P2-601

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Steroidogenesis & Its Disorders (1:30 PM-3:30 PM)

Title Long-Term Outcome in a Cohort of Adults with Congenital Adrenal Hyperplasia (CAH) Due to 21-Hydroxylase Deficiency: A Single-Center Experience

Author String LG Gomes, G Madureira, BB Mendonca, TASS Bachega
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Body Optimal clinical managements of adults with CAH are unknown. Heterogeneous cohorts using different glucocorticoid regimens did not permit to achieve conclusions about the risk of metabolic diseases, osteoporosis and infertility in CAH. **Objective:** to evaluate the clinical outcome in a large cohort of CAH patients followed in a single center. **Methods:** in this cross-sectional study, 108 CAH adults (86 F) were selected; 39 salt-wasting (SW), 41 simple-virilizing and 28 nonclassical (NC) form. Clinical form was confirmed by genotype analysis. Mean age was 27.8 ± 9.3 and 28.7 ± 12.2 yrs old in classical (CL) and NC form, respectively. Patients were initially treated with dexamethasone (DEX) 0.25 mg/d. Doses were adjusted 2x/yr to obtain normal androgen levels according to age/sex. Plasma renin activity target was the upper limit of normal range. Clinical and biochemical parameters including BMI (kg/m^2), blood pressure (BP), glucose and insulin for HOMA-IR calculation, cholesterol and triglycerides (TG) were evaluated. Bone mineral density was assessed by DXA, fertility in men by testicular ultrasound, semen analysis, testosterone (T) and inhibin B (IB) levels and in women by pregnancy rate (attempt to conceive/pregnancy success). **Results:** mean DEX doses were 0.3 ± 0.1 in CL and 0.2 ± 0.7 mg/d in NC CAH. Mean Fludrocortisone was 50 mcg/d, 18% of SW patients did not need fludrocortisone at adulthood. Overweight and obesity were found in 35.4% and 20.3% of CL, and in 32.1% and 21.4% of NC CAH, respectively. Hypertension was found in 11.3% and 10.7% of CL and NC CAH, respectively. Impaired Fasting Glucose was found in 2.8% and 7.4% of CL and NC CAH, respectively. HOMA-IR > 2.7 was found in 38% and 25% of CL and NC CAH, respectively. Diabetes was found in only one NC CAH. Cholesterol and TG levels were high in 22.9% and 18.6% in CL and in 25.9% and 11.1% in NC CAH, respectively. The prevalence of obesity, diabetes, hypertension and dyslipidemia was similar to our normal population. Mean lumbar and femoral Z score were -0.88 ± 1.2 and -0.71 ± 0.91 for CL and -0.45 ± 1.4 and 0.47 ± 1.4 for NC CAH, respectively. CL males had 33% of testicular adrenal rest tumors, which 50% were related with oligospermia, low T and IB levels. Conception attempt frequency was 50% and 70% and pregnancy rate was 90% and 93% in CL and NC, respectively. **Conclusion:** We did not observe an increase in the prevalence of adverse metabolic outcomes in CAH adults. Fertility may be compromised in CAH males.

Sources of Research Support: Grants FAPESP # 05/04726-0, CNPq # 305117/2009-2.

Nothing to Disclose: LGG, GM, BBM, TASSB

Pub # P2-602
Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Renin-Angiotensin-Aldosterone System & Hypertension (1:30 PM-3:30 PM)
Title Blinded Comparison of ^{11}C -Metomidate PET-CT Scanning with Adrenal Venous Sampling Shows High Specificity and Sensitivity for the Lateralization of Aldosterone Secretion in Patients with Primary Hyperaldosteronism and Adrenal Adenoma
Author String MJ Brown, TJ Burton, I MacKenzie, N Bird, B Koo, K Balan, M Gurnell
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Body Adrenal vein sampling (AVS) presents a considerable hurdle between diagnosis of primary hyperaldosteronism (PHA) and curative surgery. Metomidate (MTO), a potent inhibitor of the steroidogenic enzymes CYP11B1 and CYP11B2, can be $^{11}\text{CH}_3$ -labelled as a positron emission tomography (PET) tracer (1). We have measured the sensitivity and specificity of MTO scanning in patients where AVS gave a definite diagnosis of unilateral or bilateral PHA.

^{11}C -MTO was synthesised on site in the Wolfson Brain Imaging Centre using a GE Medical Systems cyclotron and automated synthesis modules. An esterification reaction used ^{11}C -iodomethane ($>750\text{ GBq}/[\mu\text{mol}]$) and the tetrabutylammonium salt of metomidate acid, (R)-(+)-1-(1-Phenylethyl)-1H-imidazole-5-carboxylic acid. This method produces ^{11}C -metomidate in radiochemical yields of $\sim 20\%$, with a purity of $>99\%$ and specific activity averaging $14.4\text{ GBq}/[\mu\text{mol}]$.

Pre-treatment with dexamethasone (D) (0.5mg qds for 3 days) was assessed in the first 6 patients, who had PET-CT scans with and without pre-treatment. In subsequent patients, scans were performed after D pre-treatment: in 19 who lateralised on AVS (aldosterone:cortisol ratio >4 on the side of an adenoma), and in four patients who were committed to adrenalectomy after failed AVS at their referring hospital. We studied 15 negative controls: five with adrenal 'incidentalomas', and 10 with bilateral PHA (<2 -fold lateralisation on AVS). All patients were injected with $150\text{-}500\text{ MBq}$ MTO and imaged for 45 minutes on a GE D690 PET CT scanner. The maximum standardised uptake values (SUV_{max}) over adenoma and normal adrenal was measured by two radiologists blinded to clinical diagnosis and AVS results.

Pre-treatment with D increased signal difference between tumour and normal adrenal by $21\pm 2.3\%$ ($p<0.05$). SUV_{max} was greater over tumour than normal in 21/23 patients who lateralised on AVS and/or subsequently had surgical cure of hypertension by adrenalectomy. At a SUV_{max} ratio of 1.25:1 between adenoma and normal adrenal, we found 87% specificity and 74% sensitivity. Several patients had adenomas only 0.5-1 cm in size. In one patient with multiple adenomas, the scan also distinguished between hot and cold nodules, as confirmed by *ex vivo* measurements of aldosterone secretion and CYP11B2 expression.

^{11}C -MTO PET-CT offers a rapid, non-invasive and accurate technique to visualise small adrenal adenomas, and differentiate functional tumours from incidentalomas.

(1) Bergstrom M et al. PET with [^{11}C]-Metomidate for the Visualization of Adrenocortical Tumors and Discrimination from Other Lesions. Clin Positron Imaging 1999; 2:339

Sources of Research Support: British Heart Foundation; Cambridge Comprehensive Local Research Network; Cambridge Biomedical Research Centre.

Nothing to Disclose: MJB, TJB, IM, NB, BK, KB, MG

Pub # P2-603

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Renin-Angiotensin-Aldosterone System & Hypertension (1:30 PM-3:30 PM)

Title A New Cost-Effective Method for Screening and Diagnosing Primary Aldosteronism

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Body Primary aldosteronism (PA) is the most common disorder that represents the secondary hypertension. The case detection test (CDT), further confirmatory test (FCT), and subtype evaluation are of particular importance for selecting an appropriate treatment strategy of the disease. The plasma aldosterone to renin activity ratio (ARR) is used as the standard CDT with following FCT. When confirmed, adrenal vein sampling (AVS) is currently the best reliable method to localize the responsible lesions. The major remaining problem is how to select or restrict AVS test recipients because of its cost, invasiveness, and requirements of highly experienced radiologists. Accordingly, FCT should be as definitive as possible; however, current methods of FCT may be time consuming, expensive, or demanding for physical stresses of the examinees. Therefore, we evaluated a new method using a combination of ARR and ACTH (cosyntropin) loading test as another CDT and/or FCT.

Consecutive 114 patients including those who were referred to our hospital because of 1) suspicion for PA having classical manifestations such as hypertension with hypokalemia, 2) PA-detecting purpose in hypertensive patients without typical manifestations of PA, and 3) further examinations for mostly incidentally identified adrenal tumor(s), were examined. ARR measurement and ACTH (cosyntropin) loading test measuring maximum value of aldosterone to cortisol ratio induced by ACTH loading (MaxACR) were performed in all 114 cases. Final diagnosis of PA (n=53) was based on 1) AVS results and pathological data in unilateral cases, 2) AVS results in bilateral or un-operated cases, or 3) biochemical confirmatory data in cases where AVS was unsuccessful or not performed.

Results: Cut-off point of [ARR>80 and/or maxACR>1.3] (n=33) yielded 62% of sensitivity and 100% of specificity for PA, indicating immediate considerations for AVS without FCT in such cases. Cut-off point of [ARR<20 and maxACR<0.8] (n=43) yielded 69% of sensitivity and 98% of specificity for non-PA. Among the remaining subjects, only 28 subjects remained as [grey zone for PA] that demanded for FCT, because [ARR>20 and maxACR>0.8] (n=10) also yielded high sensitivity and specificity for PA.

Conclusion: If ARR>20 had solely been used for CDT in our 114 subjects, 54 would have demanded for FCT as grey zone and 6 with PA would have been overlooked. Our new method improved the rates of misdiagnosis of PA and reduced the expense of diagnostic tests.

Nothing to Disclose: NM, RG, KT, DK, JK, HM, TK, SS, YS, YY, ME, HS, EA

Pub # P2-604

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Renin-Angiotensin-Aldosterone System & Hypertension (1:30 PM-3:30 PM)

Title Primary Aldosteronism: Effect of Different Protocols on Lateralization by Adrenal Venous Sampling

Author String A Tanabe, M Tsuiki, A Tsumagari, H Kimura, M Naruse, N Hizuka
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Body **Objectives:** Adrenal venous sampling (AVS) is the criterion standard test for subtype evaluation of primary aldosteronism (PA). However, across institutions and countries, multiple different protocols are used for AVS. We sought to determine if lateralization data from AVS are protocol-dependent.

Subjects and Methods: Study 1--Method for cosyntropin (ACTH) administration: 57 patients with PA were randomly apportioned to continuous intravenous ACTH infusion (DIV-group; n=20) or bolus intravenous ACTH injection (IV-group; n=37) during AVS performed in the morning. Blood samples from the inferior vena cava (IVC) and bilateral adrenal veins (AV) were obtained before and 60 min after initiation of ACTH infusion (125 [mu]g/h) in DIV-group and between 20 to 30 min after ACTH injection (250 [mu]g) in IV-group. **Study 2--Timing of AVS:** 42 patients with PA were randomly apportioned to morning (0900-1200 h) (AM-group; n=16) or afternoon (1300-1700 h) (PM-group; n=26) AVS. The ACTH bolus injection protocol was used in all Study 2 patients.

Results: Plasma aldosterone concentrations (PAC) and PAC to renin ratios were not significantly different between the patient groups in both studies. **Study 1:** The baseline and peak plasma cortisol (C) concentrations were similar in the 2 groups. There were no statistically significant differences in the success rates of cannulating both AVs in the 2 groups (80% in DIV-group and 78% in IV-group). In the patients with confirmed unilateral aldosterone-producing adenoma (APA) (19 in IV-group; 12 in DIV-group), the PACs from the APA AV and contralateral AV and the PAC/C lateralization ratios were not statistically significantly different between the 2 patient groups. The mean total time for AVS was shorter in IV-group (2.1±0.3 h) vs. the DIV-group (3.3±0.2 h). **Study 2:** The baseline and stimulated AV and IVC C concentrations were similar in the AM-group and PM-group. There were no statistically significant differences in the success rates of cannulating both AVs in the 2 groups (93% in AM-group and 100% in PM-group). In the patients with confirmed unilateral APA (15 in AM-group; 17 in PM-group), the PACs from the APA AV and contralateral AV and the PAC/C lateralization ratios were not statistically significantly different between the 2 patient groups.

Conclusions: When ACTH was used during AVS in patients with PA, the method of ACTH administration and the time of day when AVS was performed did not affect the success rate or the lateralization data.

Nothing to Disclose: AT, MT, AT, HK, MN, NH

Pub # P2-605

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Renin-Angiotensin-Aldosterone System & Hypertension (1:30 PM-3:30 PM)

Title Cosyntropin-Stimulated [supersensitive] Adrenal Venous Sampling Reveals Suppressed Aldosterone Secretion from Both Attached Non-Neoplastic Parts of Ipsilateral Adrenal Gland and Contralateral Adrenal Gland in Primary Aldosteronism Due to Unilateral

Author String R Morimoto, F Satoh, M Kudo, Y Iwakura, Y Ono, O Murakami, K Takase, S Ishidoya, Y Nakamura, H Sasano, S Ito
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Body [Background] Adrenal venous sampling (AVS) has been regarded as the most reliable method to determine laterality of aldosterone hypersecretion in primary aldosteronism (PA). While some investigators advocate that stimulation with cosyntropin might be helpful in AVS (1), others do not prefer to use because cosyntropin might induce aldosterone hypersecretion from bilateral, non-neoplastic adrenal (NNA), resulting in ambiguous results of laterality compared to those without stimulation. [Aim] To evaluate effect of cosyntropin on aldosterone secretion from NNA in patients with unilateral aldosterone-producing adenomas (APA). [Patients and Methods] Cosyntropin-stimulated 'supersensitive' AVS was performed in consecutive 10 patients (seven men, mean age 49.5) who were diagnosed as PA with both baseline and captopril-loaded aldosterone over renin ratio (ng/dl per ng/ml/h) of 272±66.7 and 205±59.5 (mean±SEM), respectively. Microcatheters were cannulated into tributaries from central veins to draw samples exclusively from APA or NNA. The ratio of aldosterone over cortisol levels (A/C) was calculated. When A/C obtained from NNA was less than that obtained from peripheral vein, we determined aldosterone secretion from the NNA was 'suppressed.' [Results] In 9 cases with unilateral tumors, AVS-based laterality agreed with CT-based laterality. In the one with bilateral tumors, AVS showed left-sided hypersecretion. While cosyntropin-stimulated AVS showed A/C sampled from drainer vein of APA was 17.6±5.11 with 6.61±1.00 -fold increase from that of peripheral vein (2.49±0.41), A/C obtained from both ipsilateral NNA of APA (1.18±0.36) and contralateral adrenal (0.978±0.11) were all below that of peripheral vein with reduction of 53.1±7.70% and 53.7±8.42%, respectively. Based on cosyntropin-stimulated lateralized ratio of A/C between APA and contralateral adrenal of 18.1±4.98, unilateral adrenalectomy was performed and showed pathological diagnosis of APA and 'paradoxical hyperplasia' within NNA using immunohistochemistry of steroidogenic enzymes. [Conclusion] Cosyntropin-stimulated 'supersensitive' AVS indicated aldosterone secretion was suppressed in both ipsilateral NNA attached to APA and contralateral NNA, suggesting it is less likely that cosyntropin might induce aldosterone hyperresponse from NNA with subsequent misleading to disappearance or reversal of aldosterone laterality obtained without cosyntropin in patients with APA.

(1) Young WF et al., Clin Endocrinol (Oxf) 2009; 70:14

Nothing to Disclose: RM, FS, MK, YI, YO, OM, KT, SI, YN, HS, SI

Pub # P2-606

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Renin-Angiotensin-Aldosterone System & Hypertension (1:30 PM-3:30 PM)

Title Superselective ACTH-Stimulated Adrenal Venous Sampling Enables Treatment of Patients with Aldosterone-Producing Adenoma by Unilateral Partial Adrenalectomy

Author String M Omura, K Makita, S Matsui, M Nagata, Y Matsuzawa, J Saito, K Yamaguchi, T Nishikawa
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Yokohama Rosai Hospital, Yokohama, Japan; Yokohama Rosai Hospital, Yokohama, Japan

Body
Introduction: Patients with aldosterone-producing adenoma (APA) have been treated by unilateral total adrenalectomy of the affected adrenal gland. APA and normal adrenal tissue attaching to it have been resected simultaneously when doing unilateral total adrenalectomy. To avoid the resection of normal part of adrenal gland, we have tried to treat patients with APA by unilateral partial adrenalectomy based on the diagnosis of APA by superselective ACTH-stimulated adrenal venous sampling (SS-ACTH-AVS).
Patients and methods: Fifteen patients with unilateral CT-detectable adrenal nodule were examined by SS-ACTH-AVS. By using Omura-Makita microcatheter, adrenal effluents were obtained after ACTH stimulation at more than 2 intra-adrenal tributary veins in each adrenal gland. APA was diagnosed when level of aldosterone in adrenal effluent obtained at one intra-adrenal tributary vein connecting to the CT-detectable adrenal nodule is >1400ng/dl, and levels of aldosterone are <1400ng/dl in effluents at the other tributaries not connecting to the nodule. Adrenal segment containing CT-detectable APA was removed by laparoscopic partial adrenalectomy.
Results: Pathological examination revealed that the adrenal nodules had been completely resected without the damage of their capsules. Complete remission of hyperaldosteronism in these patients was confirmed by various endocrinological tests.
Discussion: By SS-ACTH-AVS, we can diagnose whether or not a CT-detectable adrenal nodule causes hyperaldosteronism. By unilateral partial adrenalectomy based on the diagnosis of SS-ACTH-AVS, we can decrease the surgically resected area of normal adrenal tissue attached to APA. Moreover, by preserving the normal part of adrenal gland attached to APA, it is possible to treat the contralateral adrenal gland without inducing adrenal insufficiency even when another adrenal disease affects it.

Nothing to Disclose: MO, KM, SM, MN, YM, JS, KY, TN

Pub # P2-607

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Renin-Angiotensin-Aldosterone System & Hypertension (1:30 PM-3:30 PM)

Title In Primary Hyperaldosteronism, an Adrenal Macroadenoma on CT Scan Does Not Reliably Predict Surgically Remediable Hyperaldosteronism

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Body **Background.** Adrenal CT scanning is routinely performed in patients with biochemically confirmed primary hyperaldosteronism (PA). In general, the CT results do not carry adequate sensitivity or specificity to direct therapy, and adrenal venous sampling (AVS) is recommended. This is expected, since many aldosteronomas are < 1 cm in diameter and since adrenal hyperplasia may appear as small nodules on CT scan. Adrenal macroadenomas, however, are reliably identified on CT scan and may influence the clinical decision to perform adrenalectomy. It is not known if an adrenal macroadenoma accurately predicts surgically remediable hyperaldosteronism (SRA). **Specific Aim.** The specific aim of this study was to determine the positive predictive value of a unilateral adrenal macroadenoma in PA. **Methods.** We performed a retrospective analysis of our patients with biochemically proven PA plus a unilateral adrenal macroadenoma, who underwent successful AVS both with and without cosyntropin stimulation from 1996-2010. An adrenal macroadenoma was defined as an adrenal mass [ge] 1.5 cm in its greatest dimension on CT scan. All patients in this subgroup that were predicted to have SRA by AVS underwent adrenalectomy, and cure was confirmed postoperatively by a serum aldosterone concentration < 7 ng/dl or plasma renin activity > 2.0 ng/ml/h. Patient with AVS results indicating bilateral adrenal hyperplasia (BAH) did not undergo adrenalectomy even though the CT results indicated a macroadenoma. **Results.** 13 patients had a unilateral adrenal macroadenoma out of the 60 PA patients with successful AVS. The mean patient age of this adrenal macroadenoma subgroup was 58 y. The average adrenal macroadenoma was 2.4 cm (range = 1.5 to 3.7 cm). Interestingly, in 12 patients (92%) the macroadenoma was left-sided. Biochemical cure was confirmed in all patients who underwent adrenalectomy. The macroadenoma was functional and concordant with the side of the aldosteronoma in 7 patients (53%), nonfunctional and discordant with the side of the aldosteronoma in 2 patients (15%) and nonfunctional in 4 patients (31%) with BAH. The positive predictive value of an adrenal macroadenoma in PA is 0.53 (CI 0.34-0.86). **Conclusions.** CT identification of an adrenal macroadenoma in PA does not reliably predict that removal of the macroadenoma will effect a surgical cure; therefore AVS is required to accurately predict SRA even when a macroadenoma is present on CT scan.

Nothing to Disclose: SK, MA, BT, SS, DM, CM

Pub # P2-608

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Renin-Angiotensin-Aldosterone System & Hypertension (1:30 PM-3:30 PM)

Title Evaluation of Intima-Media Thickness (IMT) of Carotid Artery in Patients with Primary Aldosteronism

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Body [Background] Aldosterone regulates tubular reabsorption of sodium and elevates blood pressure by concomitant water retention. In addition, it directly acts to vessels and central nervous system to increase peripheral vascular resistance or cardiac output. Severe hypertension in primary aldosteronism (PA) which is often resistant to multi antihypertensive, may carry higher risk of arteriosclerosis in comparison with that of essential hypertension (E-HT). In this study, we tried to evaluate IMT of carotid artery as a marker of arteriosclerotic risk in PA patients. [Subjects/Method] PA patients (n=103) and background-matched E-HT patients (n=26; m/f=11/15) were entered to this study. All of the PA patients underwent adrenal venous sampling and were diagnosed as Aldosterone Producing Adenoma (APA) (n=43; m/f=24/19) or Idiopathic hyperaldosteronism (IHA) (n=60; m/f=18/42). The subjects underwent carotid ultrasonography and their maximum IMT was decided. Furthermore, risk reductions by PA treatment were examined in 43 PA (20 APA and 23 IHA). [Result] IMT in PA was 0.97 ± 0.35 mm, which was evidently thicker than that of age-matched normal controls. Compared with E-HT, PA tended to develop severe IMT thickness (E-HT; 0.89 ± 0.42 mm). Significant correlations between IMT and aldosterone /renin activity ratio (ARR) could not be demonstrated in analysis with all PA patients, but there tended to be correlated between them ($R^2=0.162$) in pre-menopausal, non-diabetic, non-smoking and non-obese female PA (n=23). Multivariate logistic regression analysis associating IMT (1.1 mm \leftarrow) revealed that smoking was the only significant variable. Improvement of IMT at the time of 12 months after normalization of hyperaldosteronism by surgery or antialdosterone therapy depended on an individual case. [Discussion] Increased IMT in PA led a speculation that hyperaldosteronism is one of independent risk factors of arteriosclerosis or cardiovascular disease. This study showed that IMT levels were higher in PA than in E-HT, and it suggested that the arteriosclerosis in PA might result from not a secondary action by hypertension but a direct action of aldosterone. Mechanism of the irreversibility of IMT thickness in some PA patients is open to further discussion. Because several factors including age, sex, smoking and other complications may highly affect the increase of IMT, elimination of the factors is required for precise analysis.

Nothing to Disclose: KI, YO, EH, MY, KY, SS, MM, HN

Pub # P2-609

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Renin-Angiotensin-Aldosterone System & Hypertension (1:30 PM-3:30 PM)

Title Relationship between Silent Brain Infarction and Primary Aldosteronism

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Body **Background.** The presence of silent brain infarction (SBI) increases the risk of symptomatic stroke. Patients presenting with primary aldosteronism (PA) experience more cardiovascular events than patients with essential hypertension (EH) independent of blood pressure (1). Chronic kidney diseases (CKD) is also a major risk factor of CVD (2), and we have reported the prevalence of CKD patients with PA in ENDO 2010. However, the association between SBI and PA has not been clarified. Our aim of this study is to ascertain whether aldosterone excess has a relevance to SBI.

Methods. This is a cross-sectional retrospective study. A total of 112 subjects (72 patients with aldosterone producing adenoma (APA) and 40 with EH) underwent magnetic resonance imaging (MRI) of the brain to detect SBI. All the PA patients were performed adrenal vein sampling to confirm unilateral excessive aldosterone secretion, and underwent adrenalectomy.

Results. The prevalence of SBI was 65.9 % in all subjects. The frequency of SBI with PA was significantly higher than in patients with EH. Among PA patients, the estimated GFR (eGFR) had an association with SBI. In multivariate logistic analysis, plasma aldosterone concentration (PAC) was related to SBI independently, in addition to Age. (odds ratio [95% confidence interval] for PAC:1.158 [1.023-1.310]; p<0.01, for Age:1.214 [1.044-1.409]; p<0.01)

Conclusion. Higher PAC was associated with increased prevalence of SBI. PA patients should receive active detection of SBI and more intensive preventive management.

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Nothing to Disclose: MK, FS, RM, YI, YO, OM, KM, AS, AU, KT, SI

Pub # P2-610

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Renin-Angiotensin-Aldosterone System & Hypertension (1:30 PM-3:30 PM)

Title Primary Aldosteronism and Essential Hypertension: Assessment of the Global Cardiovascular Risk at Diagnosis and Follow-Up

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Body High blood pressure represents the first cause of mortality in the general population worldwide. Aim of the study was to evaluate the cardiovascular risk according to the ESH/ESC 2007 guidelines for the treatment of hypertension, in patients with primary aldosteronism (PA) and with essential hypertension (EH), at diagnosis and at follow-up. We studied 71 PA patients (29 with aldosterone producing adenoma-APA and 42 with idiopathic hyperaldosteronism-IHA, M/F 42/29, mean age 51 ± 12 yr, BMI 27 ± 4 Kg/m²) and 80 essential hypertensives (M/F 23/57, mean age 55 ± 11 yr, BMI 31 ± 7 Kg/m²), at basal and after surgical or medical treatment. After a mean follow-up period of 2.4 years for APA patients who underwent adrenalectomy, and 5.4 years for medically treated IHA, and 4 years for EH, patients were re-evaluated to assess the cardiovascular (CV) risk.

The resolution of hypertension was observed in 48% of APA and 35% of IHA.

According to the ESH/ESC 2007 guidelines the CV risk was, respectively at diagnosis and follow-up: low in 0% and 7%, moderate in 11% and 25%, high in 55% and 47%, very-high in 34% and 21%. Moreover, while 26% of patients had a grade 3 hypertension at diagnosis, only 1% had the same grade at follow-up.

The reduction of global CV risk was more evident in APA patients than in IHA, in whom, anyway, a significant increase of HDL cholesterol was observed, together with a reduction of left ventricular cardiac mass and posterior wall thickness ($p < 0.05$). Considering the prevalence of the very high risk category, a marked reduction after treatment was observed in APA (41% vs 17%), while the reduction was less evident in IHA (30% vs 25%) and very modest in EH (16% vs 14%).

As for EH, the CV risk was at diagnosis and follow-up, respectively: low 3% vs 12%, moderate 15% vs 25%, high 66% vs 49%, very high 16% vs 14% ($p < 0.05$).

Despite the same distribution of severity of hypertension among the three groups, the global CV risk was higher in PA than in EH patients, highlighting the deleterious effects of aldosterone excess on CV risk beyond blood pressure.

In conclusion, patients with PA present a high CV risk, which is however in great part reversible after specific treatment, due both to the reduced blood pressure values and to the improvement of end-organ damage. EH patients display a less evident reduction of the CV risk after treatment due to less consistent reduction of blood pressure and to the small improvement of cardiovascular risk factors.

Nothing to Disclose: VR, FT, VdT, GA, MB, GG

Pub # P2-611

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Renin-Angiotensin-Aldosterone System & Hypertension (1:30 PM-3:30 PM)

Title Concurrent Primary Aldosteronism and Subclinical Cortisol Hypersecretion: A Prospective Study

Author String F Fallo, C Bertello, D Tizzani, N Sonino, A Fassina, M-C Zennaro, S Boulkroun, S Monticone, V Crudo, J Burrello, F Veglio, P Mulatero
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Body **Background:** Cortisol hypersecretion from an aldosterone-producing adenoma has been reported anecdotally. In the absence of overt Cushing's syndrome, the true prevalence of concurrent aldosterone and subclinical cortisol hypersecretion may be underestimated since hypercortisolism is not routinely investigated in primary aldosteronism. **Objective:** To prospectively estimate the occurrence of subclinical hypercortisolism in patients with primary aldosteronism. **Methods:** Within a large population of hypertensive patients studied over the last 2 years, 76 had primary aldosteronism and were further investigated. No patient had clinical signs of hypercortisolism. Differential diagnosis between unilateral and bilateral aldosterone hypersecretion was made by CT/MRI and/or by adrenal venous sampling (AVS) with aldosterone/cortisol ratio. Subclinical hypercortisolism was defined by failure to suppress pl. cortisol to <50 nmol/L after 1 mg-overnight dexamethasone (dex), initially used as screening test, and at least one of two abnormal tests, i.e. ACTH <2 pmol/L and ur. cortisol >694 nmol/d. **Results:** Unilateral adrenal disease was found in 34 patients (micronodular hyperplasia, n=2; adenoma, n=32). Three out of these patients had pl. cortisol >50 nmol/L after dex. Only one (M, 71 y.o.) showed low-normal ACTH (1.7 pmol/L) and mildly elevated ur. cortisol (894 nmol/d) in addition to no suppression of pl. cortisol by dex (104 nmol/L). The patient had a right 4 cm adrenal mass at CT scan, and no AVS was performed. Laparoscopic adrenalectomy was followed by short-term steroid replacement to prevent adrenal insufficiency. All criteria for adrenocortical adenoma were fulfilled at histology, with a marked prevalence of *zona-fasciculata* like cells in the tumor. *In situ* hybridization showed *CYP11B1* expression only in tumoral tissue and *CYP11B2* expression only in the peritumoral area, suggesting the co-existence of cortisol-producing adenoma and aldosterone-producing hyperplasia in the same adrenal. Restoration to normal of ACTH, ur. cortisol and pl. cortisol response to dex, as well as serum K⁺ and aldosterone normalization, was seen 6 months after surgery. **Conclusions:** Concurrent aldosterone and subclinical cortisol hypersecretion is a rare event in patients with primary aldosteronism. In case of surgery, there are important implications for the perioperative management.

Nothing to Disclose: FF, CB, DT, NS, AF, M-CZ, SB, SM, VC, JB, FV, PM

Pub # P2-612

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Renin-Angiotensin-Aldosterone System & Hypertension (1:30 PM-3:30 PM)

Title Clinical Characteristics in Elderly Patients with Primary Aldosteronism

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Body Aim of the study: Primary aldosteronism (PA) is the most common cause of endocrine hypertension. Given that the number of elderly patients with hypertension is dramatically increasing, diagnostic significance of PA in elderly needs to be established. In this study, we compared the clinical characteristics between elderly and younger patients with PA. Methods: PA (n=109) and age-matched patients with essential hypertension (EH; n=61) were subjected to the following analysis. Patients were divided into 2 groups with elderly group of patients who were equal to and more than 60 years of age (PA: n=41; EH: n=34) and younger group of patients who were less than 60 years of age (PA: n=68; EH: n=27). Clinical characteristics, including duration of hypertension, number of medication, serum potassium, prevalence of hypokalemia, PAC, PRA, aldosterone to renin ratio (ARR), positive ratio of confirmatory tests, serum creatinine, eGFR, prevalence of proteinuria and prevalence of adrenal tumor on CT were compared between the elderly and younger PA groups. eGFR before and after surgery were compared in the elderly (n=8) and younger (n=21) PA groups. In addition, eGFR and the prevalence of proteinuria in the elderly and younger PA groups of PA were compared with respective age-matched EH groups. Results: 1) Prevalence of proteinuria was significantly higher ($P<0.05$) and eGFR was significantly lower ($P<0.01$) in the elderly group than the younger group in PA. No significant difference was seen in any of other parameters between the 2 groups. 2) Although deterioration of renal function was seen in both the elderly and younger groups of PA after surgery, there was no statistical difference between the groups. 3) The prevalence of proteinuria was higher and eGFR was lower in the elderly PA group than EH group, although the difference was not significant. Renal function in the younger PA group showed no significant difference from EH. Conclusions: The present study demonstrated that early detection and appropriate treatment are important even in the elderly PA to effectively prevent the target organ damages.

Nothing to Disclose: KN, TT, KN, SK, AY, TU, TT, AS, MN

Pub # P2-613

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Renin-Angiotensin-Aldosterone System & Hypertension (1:30 PM-3:30 PM)

Title The Analysis of 68 Cases with Primary Aldosteronism in Our Hospital

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Body It is always challenging for physicians whether we should proceed to the confirmatory tests and make a decision for a surgery. Thus, we have sought a safely performable in local clinics and cost beneficial method, and found the multiple measurement of aldosterone-renin ratio (ARR) may be one of the ways. We screened hypertensive patients by ARR, and ARR >20 (or 200 with aldosterone in pg/ml) was considered as positive. We, then, performed the confirmatory tests, such as rapid ACTH stimulating, captopril challenge, upright plus furosemide, and saline infusion tests. When any of those confirmatory tests were positive, those patients undergo an adrenal CT scan as PA. When surgical treatment is practicable and desired by the patient, the distinction between unilateral (UHA) and bilateral hyper aldosteronism (BHA) is made by ACTH-loading adrenal venous sampling (AVS). We, then, finally perform unilateral laparoscopic adrenalectomy or administrated spironolactone or eplerenone in the other cases.

We retrospectively analyzed our 68 cases of PA. The mean age was 57.3±12.3 years old including 28 males and 40 females, and the mean ARR was 73.5±90.5. The sensitivity of those four confirmatory tests among 51 AVS performed cases were 94.1%, 50.0%, 84.0%, and 20.0%, respectively, and the rapid ACTH stimulating test showed the highest sensitivity in UHA. Forty-six out of 51 AVS performed cases showed plasma aldosterone concentration [ge]1,400 ng/dl in either (21 cases, 41.2%) or both (25 cases, 49.0%) side, and we calculated the lateral ratio by PAC/cortisol in higher side to one in lower side, and 14 cases showed the ratio >2.6, which we consider the laterality positive. It is revealed the mean ARR of UHA was significantly higher than one of BHA, 83.0 ± 106.5 vs. 38.5 ± 25.0, respectively.

We next analyzed the effectiveness of the therapies, adrenalectomy or medication. The adrenalectomies were performed to 14 cases (20.6%) and 45 cases (66.2%) were administrated MRBs. The ratios of cases with improvement of blood pressure or decreased antihypertensive drugs among adrenalectomized or administrated cases were 92.9 vs. 84.4% or 71.4 vs. 57.8%, respectively, and the surgical therapy was more effective than MRBs administration. In conclusion, our results revealed the multiple measurement of ARR may give us some prospect of the distinction between UHA and BHA before proceeding to the confirmatory tests. It was also revealed the surgical therapy was more effective than medication.

Nothing to Disclose: TI, KY, AY, EY, RI, YA, HO, NH, MH

Pub # P2-614

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Renin-Angiotensin-Aldosterone System & Hypertension (1:30 PM-3:30 PM)

Title Structured Follow-Up after Adrenalectomy for Aldosterone-Producing Adenoma: Defining Outcome and Function of the RAA System

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Body **Objective:** Algorithms exist for identifying primary hyperaldosteronism (PHA) but there is lack of consensus on defining outcome and structuring follow-up care after resection of aldosterone producing adenomas. This single-centre cross-sectional observational study assessed biochemical and clinical outcomes of adrenalectomy for primary hyperaldosteronism and parameters defining surgical cure.

Method: Patients with confirmed PHA and unilateral adrenal abnormality on computed tomography (CT) who underwent unilateral laparoscopic adrenalectomy during the period 2003-2010. Our clinical management algorithm involved withholding all potassium-sparing (and all antihypertensive medication if possible) post-adrenalectomy and checking blood pressure (BP), serum potassium and aldosterone:renin ratio (ARR) at follow-up. In most patients requiring continuation of antihypertensive medication at discharge, the ARR was checked before medication was restarted.

Results: 31 patients: 19 M, 12 F; age 33-59 yrs. Pre-operatively all patients were hypertensive (BP>140/90mmHg); 23/31 (74%) had low serum potassium (3.0 ± 0.45 mmol/l). ARR at presentation was 3854 ± 5705 units. Average adenoma size on CT was 1.8 ± 1.1 cm. At first post-surgical follow-up visit (4-13 weeks): all were normokalaemic without potassium-sparing medication (4.5 ± 0.5 mmol/l); systolic and diastolic BP had fallen by 27 ± 25 mmHg ($p=0.011$) and 6 ± 14 mmHg ($p=0.129$) respectively in addition to reduction in number of antihypertensive medication. 18/31 (58%) were normotensive without medication (including two patients requiring five antihypertensive medications pre-surgery); 8/31 required 1-3 fewer antihypertensive medications. 19/31 had ARR measured 2 days-13 weeks post-surgery; all ARR were ≤ 700 units with measurable plasma renin activity. Surgical cure (defined by normalization of ARR and/or BP post-surgery) was 77% (24/31). Subgroup analysis showed that APA were significantly smaller in patients cured of hypertension by adrenalectomy compared to those requiring ongoing anti-hypertensive treatment ($p=0.027$).

Conclusions: In patients with PHA we propose that anti-hypertensive medication should be discontinued post-operatively with follow-up at 6 weeks for re-measurement of BP, potassium and ARR; ARR can be checked as early as day 2 post-surgery. Cure should be defined by post-operative normalization of ARR (coupled with improved BP and potassium). Smaller adenoma size appears to be predictive of greater surgical cure.

Nothing to Disclose: LS, JGHH, PVC

Pub # P2-615
Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Renin-Angiotensin-Aldosterone System & Hypertension (1:30 PM-3:30 PM)

Title The Influence of the Intermediate Phenotype of Essential Hypertension, Non-Modulation, on Salt Sensitivity in Type 2 Diabetes Mellitus

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Body **Background:** Salt sensitive hypertension (HTN) is positively associated dyslipidemia, insulin resistance, and inflammation (1-3) and occurs frequently in type 2 diabetes (T2DM)(4). Studies demonstrate that individuals with non-modulating (NM) HTN, an intermediate-phenotype of salt sensitive HTN, have a higher cardiometabolic risk profile than other salt sensitive phenotypes, such as low-renin (LR)(3). Of interest, ACE inhibition corrects the pathophysiologic hypertensive characteristics in NM(5) and may improve cardiovascular risk factors seen in this sub-set of salt sensitive individuals with T2DM. **Objective:** 1)To compare the frequency of intermediate phenotypes of HTN in T2DM versus essential hypertension.2)To determine whether NM with T2DM have a greater cardiometabolic risk profile than LR with T2DM. **Methods:** Two groups (T2DM N=101, HTN N=710) were analyzed within the HyperPATH protocol. Phenotype studies were conducted after subjects consumed two diets: a high salt (200mmol/day) and a low salt diet (10mmol/day) for 7 days each. Participants were admitted overnight to a Clinical Research Center after each diet and measurements for plasma renin activity (PRA) and aldosterone before and after a 60 minute angiotensin II infusion (3ng/kg/min) were obtained. Plasma glucose, insulin, and lipids were obtained in the morning after an 8 hour fast. Salt sensitivity was defined as a greater than 10mmHg difference in diastolic blood pressure between the diets. Statistical analyses conducted included 1)chi-square analysis for frequency comparison, and 2)un-paired t-test for two-group comparisons of normally distributed variables. **Results:** The frequency of salt sensitivity was relatively equal between the two groups(35% T2DM, 42% HTN p=0.3). Intermediate phenotype analysis demonstrated that NM status was more frequent (37% T2DM vs. 28% HTN) and LR status less frequent (13% T2DM vs. 26% HTN, p=0.004) between the two groups. Within the T2DM population, NM had significantly higher triglycerides (p=0.007) and lower HDL cholesterol (p=0.003) compared to LR. Body mass index and age were not statistically different in NM and LR with T2DM. **Conclusion:** The frequency of NM is greater in T2DM than in HTN. Further, the frequency of LR in T2DM was significantly less compared to HTN, suggesting that salt sensitivity often described in T2DM is driven by NM. As demonstrated in HTN, individuals with NM and T2DM have an increased cardiometabolic risk phenotype compared to LR with T2DM.

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- (4) Campese VM. Salt sensitive hypertension: renal and cardiovascular complications. *Nutr Metab Cardiovasc Dis*. 1999.; 9(3):143-156.
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Nothing to Disclose: PCU, BC, JSW, AM, DM, GKA, GHW

Pub # P2-616

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Renin-Angiotensin-Aldosterone System & Hypertension (1:30 PM-3:30 PM)

Title ACE Inhibitors Reduce Albuminuria More Than ARBS in Type 2 Diabetics

Author String NA Al-Sayed, RS Zimmerman, T Gao, BJ Wells, C Yu
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Body **BACKGROUND:** Evidence is established from major clinical trials that angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) improve the surrogate markers for and reduce cardiovascular disease, renal disease and stroke. Most of the studies have shown no major difference between ACEIs and ARBS in reducing cardiovascular and renal end points. However, there is no study that directly compared ACEIs with ARBS as a class effect in type 2 diabetic population with respect to overall mortality and morbidity. This study has retrospectively compared the effects of ACEIs versus ARBS as a class effect in type 2 diabetic patients with respect to overall mortality, cardiovascular and renal events. **DESIGN AND METHODS:** A review of database from the electronic medical records was conducted. Total of 16491 type 2 diabetic patients enrolled, divided into ACEI group (n=12353) and ARB group (n= 4138). Univariable analysis was performed to compare baseline patient characteristics between the groups. Chi-square test was used for categorical outcomes then propensity of being on ACE or ARB group was calculated by multivariable logistic regression. Survival analysis was performed to evaluate ACE/ARB effects on overall survival, CAD event, renal events via Cox regression, adjusting for propensity class and other baseline variables. All statistical analyses were conducted using R 2.10.1. **RESULTS:** Among the ACEI group, the mean age was 63.74 and 56.4% were male subjects. The mean age in ARB group was 64.97 and 45% were male. Baseline demographics, biochemical analyses and blood pressure were similar. When compared for overall survival and CAD events, the difference was not statistically significant, (P-value 0.12; P-value 0.991 respectively). When compared for renal events, the model was divided into: Creatinine doubling and albuminuria. It was found that ARB increases risk of creatinine doubling compared with ACE but the difference was only marginally significant at 0.05 level (HR 1.259, 95% CI 0.959 to 1.653, P-value 0.097). In the albuminuria group, patients who received ARB had higher rate of albuminuria compared to patients who received ACEI with significant difference (HR 1.313, 95% CI 1.061 to 1.624, P-value 0.012). **CONCLUSION:** ACEIs reduce albuminuria more than ARB as a class effect in type 2 diabetic patients. The overall survival and cardiovascular events were found to be similar between ACEIs and ARBs.

Sources of Research Support: Cleveland Clinic Foundation.

Disclosures: RSZ: Speaker, Novo Nordisk; Bristol-Myers Squibb; Astra Zeneca. Nothing to Disclose: NAA, TG, BJW, CY

Pub # P2-617

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Renin-Angiotensin-Aldosterone System & Hypertension (1:30 PM-3:30 PM)

Title Suppression of the Plasma Concentration of Aldosterone and Plasminogen Activator Inhibitor Type 1 by Azelnidipine in Hypertensive Patients with Type 2 Diabetes Mellitus

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Body Accumulated evidences showed that the combination therapy by Ca^{2+} channel blockade and inhibition of renin-angiotensin II axis improved the prognosis of hypertensive patients. Recently we reported that some types of Ca^{2+} channel blockers (CCBs), including azelnidipine, may decrease aldosterone synthesis and secretion in human adrenocortical carcinoma cell line, NCI-H295R adrenocortical carcinoma cells. Therefore, we designed the clinical study to evaluate the clinical utility of azelnidipine in the hypertensive patients with type 2 diabetes mellitus. The patients with hypertension and type 2 diabetes mellitus regularly going to the outpatient clinic of the Department of Internal Medicine of the Jikei University Hospital who received a CCB other than azelnidipine were recruited. A prescribed CCB was discontinued and switched to azelnidipine (16mg daily). The effects of azelnidipine were evaluated by the blood pressure and the routine laboratory tests, including plasma aldosterone concentration (PAC) and plasminogen activator inhibitor type 1 (PAI-1), before and after switching the prescribed CCB to azelnidipine. The control of type 2 diabetes was stable according to Hb_{A1C} of studied patients throughout the study period. Although the change of the CCB to azelnidipine did not result in the significant change of the blood pressure, azelnidipine resulted in decrease of PAC without the decrease of plasma renin activity ($P < 0.05$), and PAI-1 ($P < 0.05$), one of the adipocytokines, in the overall studied patients. The changes of PAC and PAI-1 may not be correlated to the change of body mass index, indicating that the suppressive actions by azelnidipine may be attributed to the direct actions of azelnidipine on the production of such agents. In conclusion, the present study revealed that azelnidipine may exert additional beneficial actions which decrease PAC and PAI-1 in the patients with hypertension and type 2 diabetes mellitus compared with other CCBs.

Disclosures: KT: Investigator, Daiichi Sankyo. Nothing to Disclose: KI, TI, TH, MS, NS

Pub # P2-618

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Renin-Angiotensin-Aldosterone System & Hypertension (1:30 PM-3:30 PM)

Title Olmesartan Does Not Cause Aldosterone Breakthrough in Hypertensive Patients with Type 2 Diabetes Mellitus

Author String N Hiroi, M Sue, A Yoshihara, G Yoshino
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Body [Background] Activation of the renin-angiotensin system (RAS), with ensuing aldosterone excess, detrimentally affects outcome in patients with hypertension and heart failure. RAS blockade with angiotensin-converting enzyme (ACE) inhibitors or angiotensin II type 1 receptor blockers (ARBs) is beneficial in such conditions. However aldosterone breakthrough during ACE inhibitors and ARBs therapy was reported in hypertension, diabetes mellitus, and chronic renal disease. It is reported that olmesartan does not cause an aldosterone breakthrough unlike other RAS blockers.

[Methods] We prospectively studied 17 hypertensive patients with diabetes mellitus (59.7 ± 12.3 years). Patients were treated with olmesartan (20 mg/day) for 12 months. Blood pressure (BP), urinary albumin excretion (UAE), biochemical markers, plasma aldosterone concentration (PAC), plasma renin activity (PRA), angiotensin I (AngI) and angiotensin II (AngII) were measured before and at 3, 6, and 12 months of treatment

[Results] Systolic and diastolic BP were lowered significantly after treatment ($p < 0.05$). PRA was significantly increased from 1.79 ± 1.66 ng/mL/hr to 5.68 ± 6.89 ng/mL/hr ($p < 0.01$), and PAC was significantly decreased from 175.3 ± 193.7 pg/mL to 92.6 ± 39.5 pg/mL ($p < 0.01$) at 12 month after administration of olmesartan. Levels of AngI and AngII were 458 ± 604 pg/mL and 30 ± 39 pg/mL at before treatment, and 673 ± 1426 pg/mL and 100 ± 194 pg/mL at 12 month after treatment. There was no significantly different. Other biochemical markers including UAE did not differ between before and after treatment.

[Conclusion] Aldosterone breakthrough was not seen in hypertensive patients with diabetes mellitus treated with olmesartan. It is reported that olmesartan is ARB with an endogenous ACE inhibitory effect, which is exerted through an increase in angiotensin (1-7) via increasing of ACE2 expression. RAS blockade therapy using olmesartan may be effective in preventing cardiovascular injury in these patients.

Nothing to Disclose: NH, MS, AY, GY

Pub # P2-619

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Renin-Angiotensin-Aldosterone System & Hypertension (1:30 PM-3:30 PM)

Title Age-Related Changes in the Renin-Aldosterone System in Healthy Subjects

Author String R Baudrand, C Campino, M Aglony, A Martinez-Aguayo, CA Carvajal, H Garcia, R Bancalari, O Padilla, G Owen, AM Kalergis, CE Fardella
Pontificia Universidad Catolica de Chile, Santiago, Chile; Pontificia Universidad Catolica de Chile, Santiago, Chile; Pontificia Universidad Catolica de Chile, Santiago, Chile; Pontificia Universidad Catolica de Chile, Santiago, Chile; Pontificia Universidad Catolica de Chile, Santiago, Chile; Pontificia Universidad Catolica de Chile, Santiago, Chile

Body Aldosterone (SA) production and plasma renin activity (PRA) are age-dependant with more pronounced modifications at the extremes of life, but the physiologic mechanisms are not fully understood. Aim: To evaluate SA, PRA, and SA to PRA ratio (ARR) in normal subjects and their modifications with age, gender, anthropometric and biochemical parameters. Subjects and Methods: We recruited 326 healthy normotensive children, adolescents, and adults [age range 5-75 years; 61% female; Body Mass Index (BMI) 22.9 ± 4.8] without any medical or psychiatric illness. In morning blood samples we evaluated: SA, PRA, cortisol (F), cortisone (E), glucose, insulin, high sensitivity C-reactive protein (hs-CRP) and lipid profile. Insulin resistance (IR) was evaluated by HOMA Calculator 2.2. Data were analyzed with Pearson's correlation coefficient and analysis of covariance (ANCOVA). Results: We observed an inverse correlation between SA with age ($r=-0.13$, $P=0.02$) and PRA and age ($r=-0.54$, $p<0.001$), and a positive correlation between ARR and age ($r=0.43$, $p<0.001$), with no differences by gender. PRA levels were inversely associated with BMI ($r=-0.54$, $p<0.001$), systolic blood pressure ($r=-0.23$, $p<0.001$), F/E ratio ($r=-0.17$, $p=0.008$), HOMA-IR ($r=-0.28$, $p=0.001$) and triglycerides ($r=-0.30$, $p<0.001$). SA levels were not associated with anthropometric and biochemical parameters. By analysis of covariance, PRA levels were inversely associated with F/E ratio ($p=0.01$) when adjusting by age, gender, BMI and blood pressure. With this adjusted statistical model, ARR level had a positive correlation with log HOMA-IR ($p=0.02$) and F/E ratio ($p=0.04$). Conclusions: We show that in healthy subjects, ARR has a continuous increase with age mainly by decreased levels of PRA. We observed that decreasing values of PRA, but not SA levels, were associated with increased blood pressure, BMI, insulin resistance and F/E ratio. These data are supported by a regression model, showing that the decline of PRA levels are partially explained by increased serum F/E ratio, even after adjustment for confounding variables. Our results suggest that a non-aldosterone activation of mineralocorticoid receptor increases with age and could be secondary to cortisol dysregulation.

Sources of Research Support: Grants from FONDEF D08I1087, FONDECYT 1100356 and Millennium Nucleus in immunology and Immunotherapy P07/088-F.

Nothing to Disclose: RB, CC, MA, AM-A, CAC, HG, RB, OP, GO, AMK, CEF

Pub # P2-620

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Renin-Angiotensin-Aldosterone System & Hypertension (1:30 PM-3:30 PM)

Title 11 β -Hydroxysteroid Dehydrogenase Type 2 (11 β -HSD2) Activity Decreases with Age in Normotensive Subjects

Author String C Campino, A Martinez-Aguayo, M Aglony, H Garcia, R Bancalari, C Avalos, L Bolte, CA Carvajal, R Baudrand, GI Owen, AM Kalergis, CE Fardella
Pontificia Universidad Católica de Chile, Santiago, Chile; Pontificia Universidad Catolica de Chile, Santiago, Chile; Pontificia Universidad Catolica, Santiago, Chile; Pontificia Universidad Catolica, Santiago, Chile

Body The prevalence of arterial hypertension increases with age, although the cause in the majority of the patients is unknown. In a previous study, we found that impairment in 11 β -HSD2 activity resulted in inefficient conversion of cortisol (F) to its inactive metabolite cortisone (E) which may trigger hypertension via the activation of the mineralocorticoid receptor (1). Currently, no information exists in respect to 11 β -HSD2 activity through the lifetime of a normotensive healthy population. **Aim:** To investigate if 11 β -HSD2 activity is age dependant in normotensive healthy subjects. **Subjects and Methods:** We selected 83 normotensive children (5-15 year old, 61% females) and 19 normotensive adults (55-65 year old, 68.4% females) who were not receiving any antihypertensive therapy. None of the adult women were under hormone replacement therapy. Fasting serum cortisol and cortisone levels (8:00-10:00 AM, in the sitting position with at least a 15-minute rest) were measured by immunoassay and the 11 β -HSD2 activity estimated by F/E ratio. The results were expressed as median values (inter-quartile range, [Q1-Q3]). Comparison between child and adult groups was performed by the Mann-Whitney test, with statistical significance set at $p < 0.05$. **Results** Cortisol levels were lower in the children group (8.4 mcg/dl [6.3-10.4]) than in the adult (12.3 mcg/dl [9.6-14.4], $p < 0.05$). Cortisone levels were higher in children group (3.2 mcg/dl [2.8-3.7]) compared to the adult group (2.7 mcg/dl [2.5-3.1], $p < 0.05$) and the F/E ratio was lower in children group (2.6 [2.1-3.2]) compared to the adult (4.2 [3.6-5.0], $p < 0.05$). **Conclusions:** Our results show: 1.- Cortisol concentrations increase with age suggesting that there is a dysregulation in the hypothalamus-pituitary-adrenal axis. 2.- The 11 β -HSD2 activity decreases with age, an effect that may cause cortisol-mediated mineralocorticoid receptor activation. These findings may contribute to the increasing prevalence of hypertension in elderly patients.

(1) Campino et al., Endocrine 2010; 37:106-114.

Sources of Research Support: Fondecyt 1100356, FONDEF D0811087 and Nucleo Millennium on Immunology and Immunotherapy P07/088-F Chilean grants.

Nothing to Disclose: CC, AM-A, MA, HG, RB, CA, LB, CAC, RB, GIO, AMK, CEF

Pub # P2-621

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Renin-Angiotensin-Aldosterone System & Hypertension (1:30 PM-3:30 PM)

Title Inflammatory Markers in Hypertensive Children and Their Relationship with Aldosterone

Author String R Bancalari, H Garcia, C Campino, M Aglony, A Martinez-Aguayo, C Carvajal, C Loureiro, C Avalos, C Fardella
Pontificia Universidad Católica de Chile, Santiago, Chile; Pontificia Universidad Católica de Chile, Santiago, Chile

Body Prevalence of arterial hypertension is increasing in adult population. The same trend is being observed in children due to change in life style. Hypertension generated endothelial damage. Currently, no information exist in respect to endothelial dysfunction in pediatric population. On the other hand high levels of Aldosterone has been correlated with endothelial inflammation. **Aim:** To explore the levels of inflammatory markers and their relationship with aldosterone in hypertensive and normotensive Chilean children. **Subjects & Methods:** A prospective clinical study we done in 298 children from the outpatient clinics of Pontificia Universidad Catolica of Chile. Blood pressure were measured 3 times consecutively at 5 minute intervals and classified according the Fourth Report of Task Force and JNC 7 guidelines. Hypertension was defined as average systolic or diastolic blood pressure levels > 95th percentile for age, gender and height. We divided the population in 3 groups: 98 hypertensive children (HH), 102 normotensive children with hypertensive parents (NH) and 98 normotensive children and parents (NN). The groups were statistically comparable in BMI (mean 78.5 percentile) , gender(51.8% female), age (mean 12.7 years). We measured high sensitive C-reactive protein (hs CRP), tumoral necrosis factor α (TNF α), interleukin 6 (IL 6), interleukin 8(IL 8), plasminogen activator inhibitor 1(PAI 1), serum aldosterone (SA) and plasma renin activity (PRA). Results were expressed as median and were compared with Kruskal Wallis test. **Results:** In HH group we found a positive trend in PAI 1 (ng/ml) values compared with NH and NN group: 18.5;17.2;14.8 (p:0.08). We did not find differences in the others inflammatory markers between groups, hs CRP(mg/L): 0.80; 0.57;0.57. TNF α (pg/ml): 17.04; 17.38; 17.43. IL 6(pg/ml): 10.63; 11.39; 13.04 and IL 8 (pg/ml):19.3; 18.55; 19.58. SA (ng/dl) and PRA (ng/ml/hr) did not show differences between groups. 5.6;6.4;6.7 and 2.2; 2.3;2.5 respectively. SA did not show correlation with inflammatory markers. **Conclusion:** In HH group PAI 1 values showed a tendency to increase than the normotensive childrens (NH and NN). Suggesting that the endothelial damage require time to manifest. The level of this inflammatory marker is independent of SA concentration at this life stage.

Sources of Research Support: FONDECYT (1100356), FONDEF (D08I1087) and NMII (P07/088-F) Chilean Grants.

Nothing to Disclose: RB, HG, CC, MA, AM-A, CC, CL, CA, CF

Pub # P2-622

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Renin-Angiotensin-Aldosterone System & Hypertension (1:30 PM-3:30 PM)

Title Phenotypic Profile of Hypertension in Young Adults

Author String Q Shafiq, NDL Fisher
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Body **Background:** Hypertension in young adults is increasing in frequency. While classic teaching dictates that secondary causes are more common in young adults, rates of essential hypertension (EH) are progressively rising. **Objectives:** In this pilot study we investigated the characteristics of young adults with EH. We hypothesized that EH in these patients would be accompanied by physical and psychological symptoms that correlate with higher plasma renin activity (PRA). **Patients & Methods:** Our endocrine clinics database was searched for young (age <40 y) patients with pre-hypertension (BP>120/80mmHg) and hypertension (BP > 140/90 mmHg) in whom seated PRA and serum aldosterone were measured. Patients with diabetes mellitus, kidney disease, and pregnancy were excluded. Logistic regression analysis was performed using SPSS v19.0 software. **Results:** 126 patients were identified. There were 6 cases of secondary hypertension (4.7%), 3 pregnant women, and 57 patients without hormonal values. Sixty patients were included in the final analysis: 44 women and 16 men, mean age 25.3±6.5 years, mean BMI 25.7±5.6. Caucasians were 85%, blacks 10%, Hispanics 3% and Asians 2%. Mean BP was 144±18/91±12 mmHg. Cumulative incidence of psychiatric diagnosis was 33%; 25% of these patients had anxiety, 16.7% panic attacks and 8.3% were diagnosed with depression. Among all patients, palpitations were the most common presenting symptom (40%), followed by headaches (37%) and flushing (27%). Pallor (8%) and syncope (7%) were less common. Mean PRA (on uncontrolled outpatient diets) was 4.1 ± 6.4 ng/ml/hr and serum aldosterone was 16 ±12ng/dl. PRA in men was significantly higher than in women (5.1±10.4 vs. 3.7 ±4.2 ng/ml/hr; p<0.05). PRA in patients with psychological disorders was significantly higher than in patients without the diagnosis (6.4±10.2 vs. 3.0±2.7 ng/ml/hr; p<0.05). Men were diagnosed with EH at an earlier age than women ((21.6±6.2 vs. 26.6± 6.1 years; p< 0.05) and had higher alcohol intake than women (p<0.05). **Conclusions:** Essential hypertension is overwhelmingly the most common cause of hypertension in young adults. A significant proportion presents with psychological symptoms and raised PRA activity. We hypothesize that both of these findings are manifestations of higher baseline sympathetic nervous system activity in many young hypertensives.

Nothing to Disclose: QS, NDLF

Pub # P2-623

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Renin-Angiotensin-Aldosterone System & Hypertension (1:30 PM-3:30 PM)

Title Factors Associated with Renal Dysfunction and I/D Polymorphism of the Angiotensin-Converting Enzyme in Hypertensive Patients

Author String JF Vilela-Martin, LN Cosenso-Martin, RO Vaz-Melo, DD Martineli, LT Giollo-Junior, MM Borges, DR Souza, CN Cosenso, JC Yugar-Toledo
Medical School of Sao Jose do Rio Preto (FAMERP), Sao Jose do Rio Preto, Brazil

Body
Background: Chronic kidney disease is a serious public health condition. Despite its known association with hypertension and diabetes, other factors influence on it.
Objectives: To identify the influence of clinical, biochemical, target organ damage (TOD) and I/D polymorphism of angiotensin converting enzyme (ACE) in the prevalence of renal dysfunction (RD) in hypertensive patients.
Methods: Cross-sectional study with 271 patients. RD was defined by creatinine clearance (CrCl) estimated < 60 ml/min/m² by MDRD equation. The multivariate logistic regression model was applied as to identify variables from the univariate analysis that were statistically significant between groups (RD x no RD). The deviation of α at 5% with level of significance of $p < 0.05$ was adopted.
Results: The prevalence of RD in the study population was 59.2%. Individuals with RD had a higher mean age ($p < 0.001$), serum creatinine ($p < 0.001$) and urinary albumin excretion (UAE) ($p = 0.001$) when compared with those without RD. Also had higher blood pressure for systolic and average when compared to those without RD. There was no difference in the prevalence of TOD between the groups, except for the stratification of UAE ($p = 0.013$). In logistic regression, individuals with macroalbuminuria had higher risk of RD (OR = 5.47, 95% CI 1.55 to 19.24, $p = 0.008$). There was no difference between groups regarding genotypes and genotype combinations of ACE, as well as in other clinical and biochemical parameters. In the univariate analysis, age ($r = -0.367$, $p < 0.001$), creatinine ($r = -0.534$, $p < 0.001$), glucose ($r = -0.122$, $p = 0.046$), UAE ($r = -0.188$, $p = 0.002$), were associated with CrCl. However, only age ($r = -0.344$, $p < 0.001$), female gender ($r = -0.6700$, $p < 0.001$), and creatinine ($r = -0.45344$, $p < 0.001$) remained significant on multivariate analysis.
Conclusion: The renal dysfunction is highly prevalent in hypertensive patients. Age, UAE, gender and creatinine correlate to estimated CrCl and the prevalence of renal dysfunction. Adequate control of blood pressure and cardiovascular risk factors must be achieved to reduce the morbidity and mortality of these patients.

Sources of Research Support: Medical School of Sao Jose do Rio Preto (FAMERP) and FAPESP.

Nothing to Disclose: JFV-M, LNC-M, ROV-M, DDM, LTG-J, MMB, DRS, CNC, JCY-T

Pub # P2-624

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Renin-Angiotensin-Aldosterone System & Hypertension (1:30 PM-3:30 PM)

Title Ghrelin IV Modulates Baroreflex Regulation of Sympathetic Vasomotor Tone in Healthy Humans

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Body
Background: Ghrelin - an orexigenic neuropeptide derived from hypothalamic structures and the stomach - triggers the release of GH from the pituitary gland. Short term IV administration is known to lower the arterial blood pressure without baroreflex-mediated increase of heart rate. Indirect suggest a reduced cardiac and vascular sympathetic activity. The present study aimed to directly elucidate the effect of ghrelin on sympathetic outflow and cardiovascular regulation in healthy humans.
Methods: 12 healthy young men (BMI 20-25 kg/m²) were treated with a single dose either of ghrelin 2 [micro]g/kg IV or placebo (cross-over-design). Muscle sympathetic nerve activity (MSNA) was recorded continuously. 10-minute intervals before (t₁), directly (t₂) and 90 minutes (t₃) after treatment were analyzed. Blood pressure and heart rate were monitored.
Results: At baseline (t₁) all parameters were nearly equal under both conditions. Shortly after ghrelin treatment (t₂) an increase in MSNA was seen compared to placebo (32.47 vs. 28.90 bursts per minute = bpm). 90 minutes after ghrelin (t₃) MSNA returned close to baseline, while under placebo condition an increase was observed (28.40 vs. 32.7 bpm, t-test 0.047). Interaction between the factors ghrelin/placebo and time of measurement was highly significant (p=0.007, ANOVA). Diastolic blood pressure was decreased shortly after ghrelin treatment (t₂: 62.0 vs. 68.0 mmHg, p=0.087). A smaller decrease was observed after 90 minutes (t₃: 61.1 vs. 63.1 mmHg). Systolic blood pressure was not altered by ghrelin. The heart rate showed a transient increase directly after ghrelin administration (t₂: 66.8 vs. 62.1 per minute, n.s.) but a significant decrease after 90 minutes (t₂: 59.3 vs. 65.8 mmHg, p=0.033; ANOVA: p=0.000 for interaction ghrelin/placebo - time of measurement).
Conclusion: 1) High ghrelin plasma levels shortly after IV administration lower diastolic and mean arterial pressure while MSNA and heart rate are increased. This might be due to a NO-mediated vasodilatation caused by ghrelin and baroreflex-mediated stimulation of sympathetic outflow. 2) 90 minutes after ghrelin treatment MSNA and heart rate are significantly decreased compared to placebo. Mean and diastolic blood pressure is slightly decreased. These findings support the hypothesis that ghrelin shifts the central nervous setpoint of sympathetic blood pressure regulation towards a lower levels with decreased MSNA.

Sources of Research Support: DFG - Deutsche Forschungsgemeinschaft.

Nothing to Disclose: AK, FM, AI, HL, FS

Pub # P2-625

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Pheochromocytoma & Paraganglioma (1:30 PM-3:30 PM)

Title Malignant Composite Pheochromocytoma with Neuroblastoma Responsive to Cyclophosphamide, Vincristine, and Dacarbazine Chemotherapy

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Body
BACKGROUND: Composite pheochromocytoma (CP) is a rare neuroendocrine tumor defined by the presence of pheochromocytoma (PH) with other neural crest derivatives such as neuroblastoma (NB), ganglioneuroma or ganglioneuroblastoma. At least 10 cases of CP with NB are reported in the literature, however only 4 are reported with metastases. At this time little is known about the natural history or optimal treatment of CP.
CLINICAL CASE: A 35 year old male presented with acute abdominal pain. Contrast CT scan of the abdomen and pelvis revealed a 7.6 cm right retroperitoneal mass. A 24 hour urine collection showed an elevated dopamine of 907 ug/day (60-440 ug/day) and normetanephrine of 1152 ug/day (50-650 ug/day). FN/ was inconclusive, and the mass was resected. At the time of surgery, liver, and retroperitoneal metastases were discovered, and a palliative resection of the primary mass, arising from the right adrenal, was performed. Pathology of the adrenal tumor, liver, and retroperitoneal metastases revealed a malignant CP with predominantly NB component. The primary mass contained distinct areas of PH (tumor cells positive for chromogranin A and sustentacular cells staining for S100) and poorly differentiated NB with a high mitotic rate and Ki67 proliferation index. Amplification of N-myc proto-oncogene was not seen (presence of amplification is associated with advanced stage and poor outcome in young children with NB). The metastases were composed of the NB component only. After surgery, 24 hour urine catecholamines normalized; serum catecholamines showed normal epinephrine, elevated norepinephrine of 1004 pg/mL (80-520 pg/mL), and elevated dopamine of 36 pg/mL (0-20 pg/mL). Serum chromogranin A was elevated at 1921 ng/mL (0-50 ng/mL). Repeat CT scan two months later revealed a new 1.9 cm liver metastasis. Chemotherapy was initiated with cyclophosphamide, vincristine, and dacarbazine (CVD). After two cycles, the liver lesion reduced in size to 1 cm. Chromogranin A levels decreased to 197ng/ml (0-50ng/mL). Repeat serum norepinephrine, epinephrine, and dopamine levels normalized.
CONCLUSION: Malignant CP with NB is an extremely rare and potentially deadly tumor. This report describes the response of a metastatic malignant CP with NB to CVD chemotherapy. In contrast to a previous report, absence of N-myc proto-oncogene amplification did not correlate with indolent behavior in this patient (1).

(1) Comstock J et al., Am J Clin Pathol 2009; 132:69

Nothing to Disclose: AH, NA, J-PL, GW, LAL, CK, AK, BP

Pub # P2-626

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Pheochromocytoma & Paraganglioma (1:30 PM-3:30 PM)

Title Single Center Experience of a Cohort of 25 Patients with Malignant Pheochromocytoma or Paraganglioma

Author String L Fishbein, B Gakhar, KL Nathanson, D Fraker, DL Cohen
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Body Pheochromocytomas and paragangliomas (Pheo/PGL) are rare neuroendocrine tumors which are generally thought of as benign. However, up to 26% of tumors are malignant with a 50% 5-yr survival rate. Limited data exists regarding patients with metastatic disease. We performed a retrospective review of 25 patients with metastatic Pheo/PGL seen in the Penn Neuroendocrine Center between 2000 -2010. The 13 men and 12 women had a mean age of 39 ± 16 years at initial diagnosis with 9/25 primary tumors in the adrenal gland, 15/25 extra-adrenal, and 2/25 head and neck tumors. At initial diagnosis, 19 patients had hypertension; only 8 patients presented with at least 2 classic symptoms of disease (headaches, palpitations, diaphoresis). The mean time from initial diagnosis of Pheo/PGL to diagnosis of metastatic disease was 4.56 years with a range of 0 to 22 years. Seven of 25 patients had a positive family history. Of the 22 patients who had clinical genetic testing, 12 had an identified germline mutation (1 *VHL*, 1 *SDHD* and 10 *SDHB*). At initial presentation, 12/25 patients had identified metastatic disease and half of those tested (5/10) had a mutation identified, all in *SDHB*. *SDHB* mutation status was not associated with shorter time from initial diagnosis to development of metastatic disease, but was associated with a younger age at initial presentation compared to patients without an *SDHB* mutation (mean age 28 vs 47 years; $p=0.002$). Biochemical testing revealed that 78% of patients had elevated normetanephrine and 52% had elevated norepinephrine. Dopamine, although not routinely tested, was elevated in 34.7% of patients. Biochemical elevations in catecholamines did not correlate with symptoms or with the location of the primary tumor. Interestingly, 18/20 patients tested had MIBG avid disease. All patients had surgery at diagnosis. Subsequently, 14 underwent MIBG therapy, 10 had radiation treatment and 8 had CVD chemotherapy. Only 8% of the cohort (2/25) died, both within 3 years of diagnosis, and 20% (5/25) had stable disease. The disease was progressive, but indolent, in 68% (17/25) of patients who currently are living 1-10 years with metastatic disease (mean 3.8 years). In conclusion, the data confirm the need for lifetime surveillance given the potential for metastatic disease up to 22 years after initial diagnosis, and it confirms the need for early screening for *SDHB* mutation carriers given the significantly younger age at presentation.

Nothing to Disclose: LF, BG, KLN, DF, DLC

Pub # P2-627

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Pheochromocytoma & Paranglioma (1:30 PM-3:30 PM)

Title Clinical Benefits of Chemotherapy in Patients with Metastatic Pheochromocytomas and Sympathetic Parangliomas: Review of the Largest Single-Institution Experience

Author String M Ayala-Ramirez, MA Habra, NL Busaidy, ND Perrier, TA Rich, SG Waguespack, A Phan, S Patel, C Jimenez
The University of Texas, MD Anderson Cancer Center, Houston, TX

Body **Background:** metastatic pheochromocytomas and paragangliomas (PHEO/PGL) are rare tumors for which there is no cure. The first chemotherapy to treat metastatic PHEO/PGL was introduced in the late 1960s. Since then, information on clinical benefits is limited to small studies that lack of systematic follow-up. **Methods:** we describe the clinical benefits (i.e, decreased tumor size, blood pressure and pain and increased survival) of chemotherapy in patients with metastatic PHEO/PGL that were treated and/or followed in our institution from 1960-2010. **Results:** we retrospectively reviewed 128 patients with the diagnosis of metastatic PHEO/PGL. Fifty-six were treated with chemotherapy. Mean age of diagnosis was 42 years (6-70 years). The overall survival (OS) at five years was 43%. Response information was evaluated at the end of the first regimen and was available on 53 patients. Seventeen patients (32%) responded to chemotherapy. All of them were treated with a cyclophosphamide/dacarbazine based regimen. 70 % received doxorubicin and 82.3% vincristine. Nine patients had tumor size reduction, 4 had blood pressure normalization and 4 exhibited tumor size reduction, blood pressure and pain improvement. Overall survival was 74.3 months for responders and 48.8 months for non-responders (log rank-test p-value= 0.421). Thirty-two of 56 had synchronous metastases with an OS of 44.3 months, while 24/56 had metachronous metastatic disease and an OS of 118 months (log-rank test p-value < 0.001). Ten out of the 17 responders had synchronous metastatic disease with an OS of 65 months. Seven patients had Metachronous metastatic disease and the OS was 95 months (log rank test p-value =0.308). We compared the survival rates in the ten responders vs the 32 non-responders that presented with synchronous metastatic disease. Responders had significantly longer survival than those who did not respond (65 months vs 32.5 months, log rank test p-value 0.003). We analyzed their characteristics and we found that non-responders had larger tumor than responders (10.5 cm vs 5.2 cm). **Conclusions:** chemotherapy may decrease tumor size and pain; and it may improve blood pressure control in patients with metastatic PHEO/sPGL. Chemotherapy is associated with a longer survival in responders vs no responders.

Sources of Research Support: The University of Texas MD Anderson's Cancer Center core grant CA016672.

Nothing to Disclose: MA-R, MAH, NLB, NDP, TAR, SGW, AP, SP, CJ

Pub # P2-628

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Pheochromocytoma & Paraganglioma (1:30 PM-3:30 PM)

Title The Characteristics of Pheochromocytoma in Incidentally Discovered Adrenal Mass

Author String ES Hong, Y Hwang Bo, JH Kim, HJ Choi, HS Jung, SW Kim, CS Shin, SY Kim
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Body

Background Some pheochromocytomas are clinically asymptomatic and thus detected incidentally on imaging studies. However, the characteristics of pheochromocytoma in incidentaloma are still not well known. The aim of this study was to investigate the characteristics of pheochromocytoma in adrenal incidentaloma.

Methods We recruited 187 patients with adrenal incidentaloma detected by imaging studies. We analyzed the laboratory, and radiological characteristics of incidentally discovered pheochromocytoma, and also compared characteristics of silent pheochromocytoma with those of symptomatic ones.

Results Of 187 patients with adrenal incidentaloma, 19 (10%) patients were diagnosed as pheochromocytoma. Mean size of tumor was 4.7 ± 2.1 cm. Hounsfield units in precontrast CT was 31.8 ± 8.9 . All of these were larger than 2cm, and had 10 or more Hounsfield units in precontrast CT. Five patients had clinical symptoms such as hypertension, tachycardia, headache and sweating. Silent pheochromocytoma (n=14) was smaller and showed lower level of 24hour urine metanephrine than symptomatic ones (n=33). There were no differences between asymptomatic and symptomatic patients in other radiologic and pathologic findings such as hemorrhage, necrosis, cystic change or capsular invasion.

Conclusion Adrenal incidentaloma with < 2 cm in size or $[le] 10$ Hounsfield units in precontrast CT imaging was less likely to be pheochromocytoma. Silent pheochromocytoma was smaller and showed lower level of 24hour urine metanephrine than symptomatic pheochromocytoma.

Nothing to Disclose: ESH, YHB, JHK, HJC, HSJ, SWK, CSS, SYK

Pub # P2-629

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Pheochromocytoma & Paraganglioma (1:30 PM-3:30 PM)

Title Clinical Significance of the Histopathological Grading Scoring in Malignant Pheochromocytoma/Paraganglioma

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Body Objectives: Although about 10% of the pheochromocytoma/paraganglioma (PHEO) is malignant, no definite method for a differential diagnosis and prediction of the prognosis has been established. Aim of the study was to elucidate the diagnostic significance of the histological grading scoring in predicting prognosis of malignant PHEO. Subjects and Methods: Eight patients with malignant PHEO experienced between 2006 and 2010 in Kyoto Medical Center were studied. Pheochromocytoma of the Adrenal Gland Scaled Score (PASS) (Thompson LD, 2002) and Grading of Adrenal and Extra-adrenal Pheochromocytoma (GAP) score (Kimura N et al., 2008) were used as the grading scoring for the histological findings. In the PASS, it is demonstrated that score of less than 4 predicts benign nature, while score equal to or more than 4 predicts malignant nature. In the GAP score, score of 1 or 2, 3 to 6, and 7 to 10 indicate well, moderately, and poorly differentiated natures of the tumor, respectively. The tissues were examined by 2 senior pathologists to minimize inter-observer variation. Results were correlated to the clinical courses. Results: The PASS was more than 4 in all but one patient (average: 5.9; range: 1-13). The patient with the highest score of 13 showed a very rapid progression with multiple metastasis 6 months after the diagnosis and deceased 9 months later. All other patients are still alive. The patient with the longest duration of 23 yrs after the first diagnosis showed the PASS of 5. The GAP score was above 3 in all patients: 6 patients with a score of 3 to 6 (average: 4.2) corresponding to the moderate differentiation and 2 patients with a score above 7 (average: 8.5) corresponding to the poor differentiation, respectively. The patient with the highest score of 9 was the same as that by the PASS. The average duration between the first diagnosis and metastasis was 4.5 yrs in the moderately differentiated tumors and 3 yrs in the poorly differentiated tumors. The patient with the longest duration of 23 yrs after the first diagnosis showed the GAP score of 4. Conclusions: These results demonstrated that histological analyses correlated, at least in part, to the clinical course of malignant PHEO. Given that no definite biomarkers for the prediction of clinical prognosis are available, histopathological grading score could be useful in predicting the prognosis, although longer-term cohort study in a larger number of patients are required.

Sources of Research Support: Health Labour Sciences Research Grant for the Research on Measures for Intractable Diseases from Ministry of Health Labour and Welfare in Japan.

Nothing to Disclose: KN, KN, SK, AY, TT, TT, TY, SM, TU, AS, MN

Pub # P2-630
Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Pheochromocytoma & Paraganglioma (1:30 PM-3:30 PM)

Title Glucocorticoids Stimulate Phenylethanolamine *N*-Methyltransferase mRNA Expression in Pheochromocytoma Tissues and Cells: Implications for Glucocorticoid Treatment-Associated Development of Pheochromocytoma Crisis

Author String SMJ Fliedner, H Lehnert, K Pacak, T Kino
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Body In recent years, cases with pheochromocytoma (PHEO) or paraganglioma (PGL) who developed crisis during treatment with glucocorticoids have been reported (1-4). It has long been known that chromaffin cells of the adrenal medulla express glucocorticoid receptors (GR) and glucocorticoid treatment induces catecholamine synthesis. Glucocorticoid response elements have been reported for the rat tyrosine hydroxylase and phenylethanolamine *N*-methyltransferase (PNMT) genes. However, to date the direct effect of glucocorticoids on PHEO/PGL is not well established.

Here we show by quantitative real-time-PCR that GR mRNA is expressed in human PHEO/PGL tissue. We compared the mRNA expression of multiple endocrine neoplasia (MEN) derived (n=5), succinate dehydrogenase B derived (n=11), von Hippel-Lindau derived (n=5), epinephrine (n=6) and norepinephrine (n=10) producing apparently sporadic PHEOs/PGLs to normal adrenal medulla samples (n=5). GR mRNA expression was elevated in epinephrine producing sporadic PHEOs/PGLs by 4.41 ± 1.25 ($p < 0.01$) and MEN-derived PHEOs by 2.13 ± 0.24 ($p = 0.047$) relative to normal adrenal medulla tissue. In addition, mouse PHEO cells (MPC) and human primary cells of a MEN-derived PHEO express GR mRNA. Treatment of MPC and human primary PHEO cells with the synthetic glucocorticoid dexamethasone induced PNMT mRNA levels by ~1000 fold in a dose-dependent fashion, while combined treatment with the GR receptor antagonist mifpristone lead to PNMT levels comparable to that in untreated cells. This drastic increase in the PNMT level may well be responsible for the observed catecholamine crisis in PHEO/PGL patients treated with glucocorticoids.

- (1) Rashid-Farokhi F et al., Arch Iran Med 2009; 12:190
- (2) Rosas AL et al., Eur J Endocrinol 2008; 158:423
- (3) Eisenhofer G et al., Drug Saf 2007; 30:1031
- (4) Takagi S et al., Jpn Circ J 2000; 64:785

Sources of Research Support: Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD.

Nothing to Disclose: SMJF, HL, KP, TK

Pub # P2-631
Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Pheochromocytoma & Paraganglioma (1:30 PM-3:30 PM)
Title Nationwide Survey and PHEO Network for the Study of Pheochromocytoma/Paraganglioma in Japan (PHEO-J)
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Body Objectives: While most of the pheochromocytoma (PHEO) is surgically curable, malignant PHEO is an intractable disease which needs establishment of early diagnosis and effective treatments. We have organized a task force group for PHEO by Ministry of Health, Labour and Welfare in Japan. The aim of the study was the survey of PHEO in Japan. Subjects and Methods: The cross-sectional nation-wide survey on PHEO was conducted based upon the manual for the epidemiological survey of the Japan Society of Epidemiology. Number of patients experienced in the 6303 departments of 2387 hospitals was investigated. Results: Recovery of the 1st and 2nd survey was 60% and 90%, respectively. The estimated numbers of patients was 2920 in total with 320 malignant patients. Malignant, multiple, extra-adrenal, and familiar PHEO were 11%, 12.7%, 17.3%, and 10%, respectively. Those patients with malignant PHEO were first diagnosed as benign in 36.8%, adrenal lesion in 61.9%, solitary in 78.9%, and no metastasis in 59.6%, respectively. Logistic analysis showed significantly higher odds ratio (3.1) and Cox Hazard ratio (2.8) in patients with abdominal paraganglioma below the age of 45 years. In addition, 68% of the patients showed noradrenalin-dominant pattern. As the treatments of malignant PHEO, surgery was performed in 55.6%, CVD chemotherapy in 40.4%, ¹³¹I-MIBG therapy in 23.4%, external irradiation to the bone metastasis in 19.3%, and transarterial embolization to the liver metastatic lesions in 7.6%, respectively. Half of the patients were treated with a combination of two or more of treatments. Overall effects were exacerbation in 42%, no change in 26%, partial remission in 23%, and remission in 3%. In addition to the nation-wide survey, we have developed the diagnostic criteria for benign and malignant PHEO, guideline for the diagnosis and treatments, and on-line PHEO registry for a longer period of follow-up. Conclusions: Nation-wide survey of the PHEO demonstrated an estimated number of malignant patients is about 300 in Japan. One third of the patients were first diagnosed as benign, indicating the difficulties in the differential diagnosis of malignant from benign. The PHEO network and PHEO registry will provide powerful functional infrastructure for the better clinical practice and research in Japan.

Sources of Research Support: Health Labour Sciences Research Grant for the Research on Measures for Intractable Diseases from Ministry of Health Labour and Welfare in Japan.

Nothing to Disclose: MN

Pub # P2-632
Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Pheochromocytoma & Paraganglioma (1:30 PM-3:30 PM)

Title The Severity of the Phenotype Linked to a *SDHD* IVS2-1>T Mutation Is Highly Variable

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Body Germline mutations of *SDHD* predispose to hereditary head and neck paragangliomas (HNP) but also to abdominal paraganglioma (PGL) or pheochromocytoma (PHEO). In almost all *SDHD*- linked PGL families a disease occurs only after paternal transmission of the mutation (1), even if one case of PGL occurring after maternal transmission of a *SDHD* mutation has been reported (2). The *SDHD* IVS2-1G>T that induces an exon 3 skipping, is the most frequent mutation identified in a large series of patients with PGL (3). However the phenotype is detailed for only 6 patients. In this study we gathered the data obtained on 31 gene carriers evaluated in the centre by a unique investigation protocol to accurately assess the phenotypes associated with this mutation.

Patients & Methods. Fourteen families with a *SDHD* IVS2-1G>T mutation were included in the study, representing a total of 31 gene carriers (14 index patients and 17 relatives). Biological investigations: measurement of plasma free metanephrines, urinary total metanephrines, and serum chromogranin A. Imaging protocol consists of an angio CT scan of the head and neck, a CT-scan of the chest, abdomen and pelvis. All imaging data were reviewed by the same radiologist.

Results. Most of the relatives (12, 70.6%) were symptomatic. A total of 26 gene carriers were thus considered as affected patients. Their mean age at clinical onset was 38.2 (SD: 15.5); the mean number of lesions was 3.1 (SD:1.7) per patient. The tumors involved the head and neck region in 15 cases (57.7%), the adrenals in 2 cases (7.7%), both sites in 5 cases (19.2%), the chest in 3 cases (15.4%), or adrenals and chest in one case (3.8%). We focused on a kindred consisting of 6 gene carriers on 3 generations. The germline mutation was transmitted from the grand father to 2 of his sons: II.9 who developed two HNP at 50, and II.11 who developed one PHEO and an endopharyngeal PGL at 47. At the 3rd generation, 3 of the 4 gene carriers became symptomatic earlier between 16 to 21, and presented from 2 to 8 tumors (HNP, PHEO and/or abdominal PGL).

Discussion. We show that the risk of developing a PHEO in the context of a *SDHD* IVS2-1G>T mutation is high (30.7%), and that there is a trend toward a genetic anticipation in *SDHD*-linked PGL as previously suggested (4). Therefore we recommend that (i) imaging screening by non invasive methods begin early in the relatives; (ii) all gene carriers undergo biological and imaging screening to identify a secreting tumor.

(1) Hensen EF et al., Eur J Hum Genet 2010; 18:62-

(2) Pigny P et al., J Clin Endocrinol Metab 2008; 93:1609-

(3) Burnichon N et al., J Clin Endocrinol Metab 2009; 94:2817-

(4) Baysal BE et al., Am J Hum Genet 1997; 60:121-

Nothing to Disclose: CC-B, CV, MK, BC, M-CV, J-LW, PP

Pub # P2-633

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Pheochromocytoma & Paraganglioma (1:30 PM-3:30 PM)

Title Experience with the Tyrosine Kinase Inhibitor Sunitinib in Metastatic Pheochromocytomas and Paragangliomas

Author String M Ayala-Ramirez, MA Habra, TA Rich, GJ Cote, NL Busaidy, P Anderson, SG Waguespack, A Phan, S Patel, C Jimenez
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Body
Background: Sympathetic pheochromocytomas/paragangliomas (PHEO/PGL) are rare tumors for which there is no curative treatment. Few reports have suggested that sunitinib, a multi-targeted tyrosine kinase inhibitor, may benefit patients with metastatic PHEO/PGL.
Methods: We describe our institutional experience from 2007 through 2010 with the off-label use of sunitinib in patients with metastatic PHEO/PGL.
Results: seven women and four men were treated with sunitinib at a median age of 55 years (range 13-69 years). All patients had genetic testing. Four had germline mutations of the *SDHB* gene and one of the *VHL* gene. Seven tumors were PHEOs and four were PGLs. Median primary tumor size was 7.7 cm. Four patients exhibited the following responses to sunitinib: reduced tumor size, decreased catecholamine and iodine I-131 metaiodobenzylguanidine uptake, and decreased pain and/or blood pressure lowering. Of these four patients, one had tumor progression 9 months after initiating sunitinib; the second patient responded initially but discontinued treatment after 1 year because of kidney insufficiency, after which the disease rapidly progressed; the third patient has been receiving sunitinib treatment for 18 months with radiographic and symptomatic improvement. The fourth patient has had improvement in adrenergic symptoms and has decreased metabolic activity in metastatic lesions as demonstrated by ¹⁸F-FDG-PET scan. Three other patients did not tolerate treatment because of early adverse effects (skeletal metastases pain, fatigue, and syncope). The remaining four patients had progression of disease after treatment.
Conclusions: Patients with metastatic PHEO/PGL may benefit from sunitinib, with tumor size reduction and symptomatic improvement. Dosage titration may be required to improve or prevent adverse events. In some patients, tumor drug resistance may occur. Upon discontinuation, responders may exhibit disease exacerbation.

Sources of Research Support: The University of Texas MD Anderson's Cancer Center core grant CA16672.

Nothing to Disclose: MA-R, MAH, TAR, GJC, NLB, PA, SGW, AP, SP, CJ

Pub # P2-634

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Pheochromocytoma & Paraganglioma (1:30 PM-3:30 PM)

Title Peptide Receptor Radionuclide Therapy of a Patient with Metastatic Cervical Paraganglioma Due to a Novel SDHD Gene Mutation (H102R)

Author String TD Poeppel, A Yucee, C Boy, E Kaminsky, A Bockisch, K Mann, LC Moeller
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Body **Background:** Paragangliomas are rare neuroendocrine tumors and can occur sporadically or as part of hereditary paraganglioma-pheochromocytoma syndromes. Treatment options of metastatic disease are limited and include external radiation, peptide receptor radionuclide therapy (PRRT) and chemotherapy.
Case report: We report a case of a 24 y/o woman presenting with loss of weight (15kg in 6 months) and a history of carotid paraganglioma resection at age 16. Ultrasound and MRI showed disseminated bone and liver metastases. Laboratory analyses found elevated levels of chromogranin A (732[μg/l, nl 19-98) and serotonin (500[μg/l, nl <250). Plasma and 24-h-urine nor-/metanephrine were not elevated. Hepatic histology confirmed metastatic paraganglioma and was congruent with that from the initial surgery. Ki-67 expression was about 3%. Metastatic disease is more prevalent in carriers of succinate dehydrogenase (SDH) B gene mutations, yet we found no mutation in SDHB. Further sequencing revealed a novel mutation in exon 3 of the SDHD gene (c.305A>G; p.H102R). Staging with ⁶⁸Ga-DOTATOC-PET/CT showed remarkably high lesional uptake (SUVmax 149) and additionally revealed local relapse. Metastases were negative for ¹²⁴I-MIBG. PRRT with ⁹⁰Y-DOTATOC was performed. Staging after 3 months showed stable disease and a second cycle was applied. Re-Staging after 3 months showed minor response. Both treatments were well tolerated. No kidney toxicity occurred.
Conclusion: We present a patient with PGL1 and initially a solitary carotid paraganglioma due to a novel SDHD mutation (H102R). Carriers of SDHD mutations often develop multiple benign tumors and metastatic disease is rare, yet this patient presented with bone and liver metastases seven years after paraganglioma resection. The metastases showed exceptionally high binding of DOTATOC but negligible binding of MIBG. PRRT with ⁹⁰Y-DOTATOC was performed and well tolerated. Until now, therapy achieved a minor response

Nothing to Disclose: TDP, AY, CB, EK, AB, KM, LCM

Pub # P2-635

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Pheochromocytoma & Paraganglioma (1:30 PM-3:30 PM)

Title Do Size and Functional Status Determine ¹²³I-Metaiodobenzylguanidine Uptake by Mediastinal Paraganglioma?

Author String S Subbarayan, M Hamaty
Cleveland Clinic, Cleveland, OH

Body Paragangliomas are rare tumors of neuroendocrine origin, accounting for 0.3% of mediastinal neoplasms. Catecholamine over-secretion is common in these tumors. MIBG scan helps further characterization of paraganglioma and searching for other sites of the disease. MIBG scan has been reported to have high specificity and lower sensitivity in identifying paraganglionoma.
CASE: A 44-year old female was diagnosed with a vascular mediastinal mass adjacent to left atrium in the course of an evaluation for chest pain and dyspnea. The mass was suspected be a hemangioma. Patient had supraventricular tachycardia treated with ablation 4 years ago with no recurrence of palpitations, postural dizziness or syncopal episodes. Hypertension had been treated with HCTZ and amlodipine for 10 years. A month prior to admission, regimen was changed to metoprolol. She maintained adequate BP control. There was no family history of endocrine tumors. Her mother and one cousin had sudden cardiac death at age 40. Attempted surgical resection was aborted because of hypertensive crisis during induction of anesthesia. Subsequently, evaluation for pheochromocytoma was initiated. Plasma free normetanephrine was 4.38 nmol/ml (N <0.9). Clonidine suppression test was performed. Dopamine and norepinephrine levels were 353 (N <20), 2383(N 80 - 520) pg/ml respectively at baseline and 376, 2540 pg/ml after clonidine. Epinephrine level was <10 pg/ml (N 10 - 200) before and after clonidine. CT chest confirmed 4 x 3 x 2 cm mediastinal mass. CT abdomen was normal. No lesions were identified on MIBG scan done after holding metoprolol for 2 weeks. Patient underwent resection of the mass after preoperative preparation with phenoxybenzamine and propranolol. Patient did well after surgery with good BP control on low dose metoprolol. Histopathology showed a paraganglioma with margins negative for tumor, with no necrosis or hemorrhage. Genetic counseling was provided and testing for SDH mutations was recommended.
CONCLUSION: Sensitivity of the MIBG scan has been reported to be higher for adrenal pheochromocytomas compared to paragangliomas. It does not seem to be dependent on tumor size or the functional status.¹In our case, there was no necrosis or hemorrhage in the tumor to explain the negative MIBG. Oversecretion of dopamine might explain atypical presentation with easy to control hypertension. Further follow up of this patient should include periodic biochemical evaluation of catecholamine secretion.

(1) Bhatia KS et al. Clin Endocrinol (Oxf) 2008 Aug;69(2):181-8. Epub 2008 Apr 3.

Nothing to Disclose: SS, MH

Pub # P2-636

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Pheochromocytoma & Paraganglioma (1:30 PM-3:30 PM)

Title Mediastinal Paraganglioma: The University of Texas MD Anderson Cancer Center (MDACC) Experience

Author String JM Varghese, M Ayala-Ramirez, A Vaporciyan, Z Liao, MA Habra, C Jimenez
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Body

Background: Mediastinal paragangliomas are rare tumors of the autonomic nervous system. Very little data is available on the clinical and therapeutic outcomes. We report a review of 14 patients with mediastinal paraganglioma seen at MDACC.

Methods: Retrospective review of medical records. Data obtained were reviewed to determine clinical presentation, extent of disease and treatment modalities.

Results: We identified 110 sympathetic paraganglioma cases (1960-2010). Fourteen (12.7%) were mediastinal. Diagnosis was confirmed by pathology. Median age at presentation was 46 years (26-70). Ten (71.4%) were male. Six patients presented with adrenergic symptoms. Eight patients were diagnosed incidentally. Hormone testing was available for 8/14 patients and catecholamines were elevated in 4 patients. The hormones produced in excess were norepinephrine and dopamine. The median tumor size was 4.8 cm (3-13). Nine patients had metastases (64%) (5 synchronous, 4 metachronous). The median time to development of metachronous metastases was 36.38 months (28-116). The overall survival for patients with metastatic paragangliomas was 140 vs. 217 months for those without metastases. Eleven patients received surgery. Five patients had external beam radiation (XRT) for unresectable disease, residual disease after surgery or for symptom control (pain, hemoptysis). Disease did not recur in the primary location in two patients who had surgery followed by XRT (positive surgical margins). Two patients with unresectable tumor had size reduction and symptom control after XRT. Six patients also received chemotherapy for progressive or metastatic disease. One of these patients had complete resolution of metastatic lesions. One patient had resolution of adrenergic symptoms.

Conclusion: Mediastinal paragangliomas are rare sympathetic paragangliomas. When hormonally active they secrete norepinephrine and/or dopamine. Frequently, they are locally invasive and associated with synchronous and/or metachronous metastases. These patients need a long-term follow up. Surgery is offered as primary method of local control when feasible. Adjuvant XRT may provide improved local control. Unresectable tumors may achieve palliation with XRT.

Nothing to Disclose: JMV, MA-R, AV, ZL, MAH, CJ

Pub # P2-637

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Pheochromocytoma & Paraganglioma (1:30 PM-3:30 PM)

Title Normotensive Extra-Adrenal Pheochromocytoma Associated with Systemic Amyloidosis -- Casual or Causal Association?

Author String M Tucci, SL Batista, S Tucci, Jr, GE Barros Silva, J Elias, Jr, M de Castro, AC Moreira, PCL Elias
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Body Background: Pheochromocytoma is a rare tumor usually causing arterial hypertension. It can occur sporadically or be part of well described syndromes such as endocrine neoplasia type 2 (MEN 2A), von Hippel-Lindau and neurofibromatosis type 1. One fifth of cases may present as normotensive. Case Report: A 66 years old woman was referred due to an incidental para-adrenal mass found during work-out diagnostic investigation of AA-type amyloidosis (secondary amyloidosis) confirmed by cutaneous and kidney biopsies. She had never experienced arterial hypertension or other paroxysmal symptoms. Patient's clinical manifestations of amyloidosis included myopathy, cutaneous manifestations, proteinuria and cardiomyopathy with heart failure. During inward hospitalization there was progressive deterioration of cardiac function with echocardiogram features typical of cardiac amyloidosis. Initial tests investigation of the para-adrenal incidentaloma revealed sustained high plasma epinephrine 2073 and 2913pg/ml (NR: 112-658 pg/ml) and urinary normetanephrine 2122 and 2606ug/24h (NR:52-310ug/24h). Imaging studies showed a heterogeneous 4.0x3.9cm para-adrenal mass, attenuation coefficient of 36 Hounsfield units (HU) on CT, and a positive uptake after 131-MIBG. Preoperative α -blockade was instituted with prazosin with progressive doses up to 8mg/day/2 weeks and the patient underwent an uneventful surgery excision of the extra-adrenal mass. However, postoperatively the patient developed a severe refractory heart failure with fatal clinical course. Anatomopathological aspects were consistent with extra-adrenal pheochromocytoma and necropsy findings confirmed amyloid deposition in various organs including heart, kidney, liver, muscles, and also in the tumor. Conclusions: adrenal and retroperitoneal incidentalomas with HU>10 on CT should be investigated to rule out pheochromocytomas, even in a normotensive patient. We advocate that preoperative alpha-blockade should be performed also in normotensive pheochromocytomas. Systemic amyloidosis can be a complication of malignant neoplasm such as renal cancer or Hodgkin disease. Although cutaneous lichen amyloidosis has been described in MEN 2A families harbouring RET proto-oncogene mutation in codon 634, the present case is the first description of association between extra-adrenal pheochromocytoma and systemic amyloidosis. Whether the present association is spurious or has a cause-effect link is yet to be determined.

Sources of Research Support: FAPESP Grant: 07-58365-3.

Nothing to Disclose: MT, SLB, ST, GEBS, JE, MdC, ACM, PCLE

Pub # P2-638

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Pheochromocytoma & Paraganglioma (1:30 PM-3:30 PM)

Title Noncardiogenic Pulmonary Edema in a Patient with Pheochromocytoma

Author String KJ Rosenquist, RG Dluhy, AR Saxena
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Body
Background: Several case reports document cardiogenic pulmonary edema resulting from pheochromocytomas. Here we report a case of noncardiogenic, bilateral alveolar pulmonary edema in a young man with pheochromocytoma.
Clinical Case: A 19-year-old, healthy male presented with intractable vomiting and a history of intermittent headaches and palpitations. Evaluation revealed a heterogeneous 4.0 x 3.5 cm right adrenal mass on abdominal CT and markedly elevated plasma normetanephrine 28.8 nmol/L (0.00-0.89) and normal plasma metanephrine 0.47 nmol/L (0.00-0.49). The patient had a complicated hospital course with sustained hypotension secondary to cardiogenic shock in the setting of markedly reduced LVEF of 17%, requiring support by an intra-aortic balloon pump and a ventricular assist device. Eventually, his hemodynamic status stabilized off of all support devices. With recovery of his cardiac function, he became frankly hypertensive with poor response to alpha blockade. Ultimately, he required a combination of phentolamine, doxazosin, phenoxymethamine and continuous intravenous diltiazem in preparation for scheduled adrenalectomy. While awaiting surgery, his respiratory status acutely deteriorated and a chest x-ray showed the development of acute bilateral, diffuse alveolar edema. An immediate cardiac echocardiogram showed normal global function (LVEF 60%). He underwent emergent laparoscopic right adrenalectomy without complications. Post-operatively, he was extubated within 18 hours and his chest x-ray showed complete resolution of the alveolar edema.
Clinical Lessons: Pheochromocytomas may be related to acute bilateral noncardiogenic pulmonary edema and should be considered as a source of acute respiratory decompensation in any patient with a known adrenal mass.
Conclusion: This case represents one of the few documented clinical cases of acute bilateral alveolar edema associated with pheochromocytoma in the setting of normal cardiac function. We believe that the patient's worsening respiratory status was due to the direct effects of excess catecholamines on pulmonary vascular permeability, resulting in acute, rapidly progressive, alveolar edema. Animal studies of acute sympathetic activation demonstrate flash pulmonary edema and alveolar flooding resulting from the effects of excess catecholamines on pulmonary capillaries. In these studies, as in our case, pulmonary edema immediately resolves with removal of the source of excess catecholamines.

Nothing to Disclose: KJR, RGD, ARS

Pub # P2-639

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Pheochromocytoma & Paraganglioma (1:30 PM-3:30 PM)

Title Atypical Clinical Features of a True Pheochromocytoma: Is There "Subclinical" Pheochromocytoma?

Author String J Miragaya, K Ramesh, O Obi, M Bermann
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Body

Introduction: The widespread use of diagnostic imaging has resulted in a vast increase in adrenal incidentalomas. A biochemical workup is recommended to identify functional tumors and to guide further management. Pheochromocytoma is a rare neuroendocrine tumor occurring in 0.05%-0.1% of the population, and 4-7% of adrenal incidentalomas are pheochromocytomas. Blood pressure (BP) control is essential for preoperative management and is achieved by alpha-blockers, followed by beta-blockers (BB). Beta-Blockers alone should induce unopposed alpha stimulation leading to hypertensive crisis. We report a case of incidentaloma/pheochromocytoma with blood pressure controlled solely with BB.

Clinical case: 61 year-old AAM presented for assessment of a 4.8 cm right adrenal incidentaloma. History included hypertension, controlled on atenolol 100 mg daily, intermittent palpitations, abdominal pain and increased breasts size. Pertinent physical findings included controlled BP and gynecomastia. Laboratory studies showed: plasma normetanephrine 1028 ng/ml (0-145) and plasma metanephrine 237 pg/ml (0-62). 24 hour urine panel: normetanephrine 2017 (110-1050), metanephrine 802 (35-460), epinephrine 18 (0-20), norepinephrine 76 (0-135) and dopamine 583 (0-510). Other laboratory studies including, 1 mg dexamethasone suppression test, renin, aldosterone, estradiol, and dehydroepiandrosterone sulfate were within the normal range.

Abdominal CT scan showed a right adrenal mass measuring 4.8 x 4.2 cm and 38 Hounsfield units. MRI of the abdomen revealed a 4.4 x 4 cm heterogeneous lesion in the right suprarenal region. Medical management for preoperative BP control was initiated with phenoxybenzamine rapidly titrated to 30 mg twice daily in addition to continuation of atenolol. Patient underwent laparoscopic surgical removal of the right adrenal gland. Histopathological examination revealed pheochromocytoma. BP remained controlled postoperatively on atenolol 100 mg daily.

Conclusion: Control of BP is essential for perioperative management of pheochromocytomas. Endocrinology guidelines recommend alpha-blocker medications, such as phenoxybenzamine as the initial medication followed by BB. Interestingly, this patient was only on beta-blockers for years and did not develop hypertensive crisis. Some studies suggest that pheochromocytomas releasing dopamine might have antiadrenergic effects. Therefore, patients might present with normal BP or controlled hypertension requiring few medications.

(1) Awada SH et al., South Med J 2003; 96:914-917
(2) Eisenhofer G et al., Drug Saf 2007; 30:1031-1062
(3) Nieman LK et al., J Clin Endocrinol Metab 2010; 95:4106-4113

Nothing to Disclose: JM, KR, OO, MB

Pub # P2-640

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Pheochromocytoma & Paraganglioma (1:30 PM-3:30 PM)

Title Medical Management of Pheochromocytoma in a Pregnant Patient with MEN2A

Author String S Narayana, P Otterbeck, A Gliwa
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Body

Introduction: We present the diagnosis, clinical course, and successful medical management of pheochromocytoma in a patient with MEN2A in late pregnancy.

Clinical case: A 33-year-old black woman at 36 weeks of gestation was admitted by the obstetrics clinic for possible MEN2A based on previous laboratory assessment. She complained of a year's duration of episodic palpitations, headaches, and diaphoresis. On further assessment, she had a family history of thyroid cancer (in her mother and several aunts). Her mother was reported to have had [ldquo]adrenal surgeries[rdquo] in the past. The patient's oldest child also was diagnosed with medullary thyroid cancer (MTC) a year ago and had total thyroidectomy. A repeat laboratory assessment of the patient revealed elevated serum calcitonin (1906 pg/ml, n<10), parathyroid hormone (98.4 pg/ml, n<65), 24hr urinary metanephrines (1481 mcg/24hr, n<785) and normetanephrines (496mcg/24hr, n<375). The thyroid ultrasound showed bilateral nodules with microcalcifications, and MRI abdomen with contrast showed right adrenal adenoma with increased intensity on T2 weighted images. While the patient was admitted, she developed mild hypertension which was successfully managed with oral phenoxybenzamine 10 mg q12h, and reactive sinus tachycardia with propranolol 20 mg q6h. After three days of medical management and observation with normal fetal heart tracings, the patient underwent C-section under combined spinal and epidural anesthesia. She gave birth to a healthy female baby weighing ~ 3 kgs. The patient was monitored in ICU setting. During the C-section, patient also underwent US-guided thyroid nodule biopsy, which was reported to be MTC. RET proto-oncogene analysis was later found to be positive. The patient was eventually discharged on phenoxybenzamine and propranolol with excellent control of blood pressure and heart rate. Further arrangements for right adrenalectomy and total thyroidectomy as well as parathyroidectomy were made on an outpatient basis. As this is an autosomal dominant condition, she was instructed to have all her 6 children evaluated for MEN2A.

Conclusion: It is crucial to recognize the possibility of pheochromocytoma as part of MEN2A in pregnancy. Its careful pharmacological management perinatally leads to a favorable course and outcome as in our patient.

Nothing to Disclose: SN, PO, AG

Pub # P2-641

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Pheochromocytoma & Paranglioma (1:30 PM-3:30 PM)

Title SDHB-Related Renal Cell Carcinoma Followed by Paranglioma

Author String T Prodanov, SW Lim-Uy, JA Coleman, KT Adams, K Pacak
NICHD/NIH, Bethesda, MD; Memorial Sloan-Kettering Cancer Center, New York, NY

Body Background: It is well known that succinate dehydrogenase subunit B (SDHB) mutations mainly predispose to extra-adrenal, and to a lesser extent, adrenal paragangliomas, with a high malignant potential, but also head and neck paragangliomas. Here we present a patient with SDHB mutation who developed renal cell carcinoma, 10 years prior to paraganglioma.
Clinical case: A 18-year man presented at the urgent care center with hematuria. Renal ultra sound revealed a large mass in the left kidney at approximately 10 x 9 x 9 cm. The patient underwent a left radical nephrectomy in November 2000. Surgical pathology revealed renal cell carcinoma, clear cell type.
Ten years later, at the age of 28, the patient developed significant weight loss, recurrent headaches, palpitations, chest pain, fatigue and increased anxiety. CT and MRI revealed a mass inferior to the bladder measuring 5.6 x 3.2 cm. Biopsy of the mass was performed with pathology findings of paraganglioma.
Biochemistry results revealed elevated plasma norepinephrine (10.2 nmol/l, URL: 4.43 nmol/l) and metanephrine (4.14 nmol/L, URL: 0.9 nmol/L). The patient underwent robotically-assisted laparoscopic partial cystectomy and resection of the pelvic mass in December 2009. The surgical pathology was consistent with a periprostatic and perivesical paraganglioma. Genetic testing revealed heterozygous for the c.600G>T, p.Trp200Cys variant in exon 6 sequence variant in the SDHB gene.
Conclusion: In some patients SDHB-related renal cell carcinoma may develop a long time before paraganglioma occurs.

Nothing to Disclose: TP, SWL-U, JAC, KTA, KP

Pub # P2-642

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Pheochromocytoma & Paraganglioma (1:30 PM-3:30 PM)

Title Acute Renal Failure from Retroperitoneal Paraganglioma

Author String S Radhakrishnan, G Sun, M Li-Ng, B Hatipoglu
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Body **Background:** Paragangliomas of the retroperitoneum are rare tumors arising from extra-adrenal chromaffin cells. Most retroperitoneal paragangliomas are functional and often present with signs and symptoms of catecholamine excess. We report a case of acute renal failure due to uncontrolled hypertension in a male who was otherwise asymptomatic from his paraganglioma.
Case: A 42-year-old African American male presented with bilateral flank pain for three days and syncope. He denied any headaches, diaphoresis, palpitations, or weight loss. His past medical history included hypertension, stage 3 chronic kidney disease and sickle cell anemia. His blood pressure was 250/160 mm Hg with a pulse of 68 beats/minute. Physical exam was otherwise unremarkable. His serum creatinine was 5.0 mg/dl (baseline 3.1 mg/dl, nl 0.7-1.4 ng/dl), serum potassium was elevated at 6.5 mmol/L (nl 3.5-5.0 mmol/L). Five months prior, he had a similar presentation for uncontrolled hypertension with seizures despite being on metoprolol, hydralazine and amlodipine. At that time, his plasma norepinephrine level was elevated at 10,020 pg/ml (nl 80-520 pg/ml) while his plasma epinephrine level was undetectable. His 24-hour urine total metanephrine level was 2966 ug/24 hr (nl 140-785 ug/24 hr). CT scan of the abdomen showed a 3.2 x 2.1 cm retroperitoneal mass. He was unfortunately lost to follow-up. His metoprolol was continued. When he returned to the hospital, his plasma norepinephrine level was higher at 25,248 pg/ml with a 24-hour urine total metanephrine level of 4045 ug. His MIBG scan showed diffuse uptake in the bilateral lungs of indeterminate significance but there was no uptake at the retroperitoneal mass. However, MRI of the abdomen without contrast showed a well-defined 4.1 x 2.8 x 2.4 cm retroperitoneal lesion abutting the aorta at the level of L4 vertebral body. Diagnosis of paraganglioma was made based on the symptomatology, laboratory parameters and the location of the mass. Metoprolol was discontinued and he was started on phenoxybenzamine and nicardipine. Excision of the retroperitoneal mass has been planned.
Conclusion: This case highlights the importance of screening for catecholamine secretion as a cause of uncontrolled hypertension and the significance of being on the appropriate antihypertensive therapy. Metoprolol should have been discontinued upon discovery of his elevated catecholamine levels to avoid unopposed alpha-adrenergic receptor stimulation.

Nothing to Disclose: SR, GS, ML-N, BH

Pub # P2-643

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Pheochromocytoma & Paraganglioma (1:30 PM-3:30 PM)

Title A Case of Pheochromocytoma Complicated by Autoimmune Thyroiditis

Author String CL Vaz, H Huey
New York Downtown Hospital, New York, NY; New York Downtown Hospital, New York, NY

Body
Introduction: Coincidental involvement of adrenal and thyroid glands is well recognized. There is no established epidemiological association between pheochromocytoma and thyroiditis. Few reports have described coexistence of pheochromocytoma and autoimmune diseases including autoimmune thyroiditis and slowly progressive type 1 diabetes(1-3). We describe a 50 y/o male with hypertension presenting with a 10cm adrenal incidentaloma who developed autoimmune thyroiditis.
Case report: Adrenal incidentaloma was described as multilobulated adrenal adenoma on CT. The patient denied symptoms suggestive of pheochromocytoma or thyroid disease. NormetanephrineU24h 1746ug/24h (110-1050), metanephrineU24h 513ug/24h(35-460), renin 5.35ng/ml/hr(15-3.95), aldosterone 17.7 ng/dl (1-16), DHEAS 251ug/dl(70-310), Negative dexa suppression; TSH 3.09uIU/ml(.35-5.5), T4 8.9ug/dl(4.5-12), T3U 32%(24-39), dopamine <10pg/ml(0-30), norepinephrine 600pg/ml(70-750), epinephrine 122pg/ml (0-110) and equivocal clonidine suppression. Resection was performed, biopsy revealed pheochromocytoma. 2 weeks later the patient had tender thyromegaly. Work up revealed initial thyrotoxic release followed by hypothyroid phase, TSH 0.03, then 76.34(0.4-5.5), total T3 237ng/dl(60-181), FT4 6.31units(1.53-3.85), total T4 17.1ug/dl then <0.5(4.5-12), T3U 36.9% then 24%(27.8-40.7), thyroid peroxidase AB>70IU/ml(0-2), thyroglobulin AB>90IU/ml(<2). The presence of thyroid peroxidase antibodies suggested Hashimoto thyroiditis. Radionuclide scan was consistent with hypothyroidism from thyroiditis. Thyroid panel normalized with hormone replacement.
Discussion: This case exhibited a rare combination of pheochromocytoma and Hashimoto thyroiditis presenting post resection. Previous reports have described a coexistence of pheochromocytoma and various forms of thyroiditis including chronic and autoimmune(1-3). Thyroiditis presented before pheochromocytoma was detected in previous reports contrary to this case. Studies describe a role of catecholamines in immune system regulation. INF γ and other Th1 cytokines contribute to the pathogenesis of autoimmune diseases(1). β 2-agonists inhibit INF γ production by Th1 cells(4). Tumor derived catecholamines could have presumably contributed to the pathogenesis of the autoimmune thyroiditis. Screening for thyroid antibodies should be considered in patients with pheochromocytoma. Further studies on possible association between autoimmune thyroid disease and pheochromocytoma are needed.

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Nothing to Disclose: CLV, HH

Pub # P2-644
Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Pheochromocytoma & Paraganglioma (1:30 PM-3:30 PM)
Title Mediastinal Paraganglioma during Pregnancy
Author String M Kawji, M Trabolsi, T Yasmeen, F Hasan
University of Illinois at Chicago/Advocate Christ Medical Center, Oak Lawn, IL; University of Illinois at Chicago/Advocate Christ Medical Center, Oak Lawn, IL

Body **Background:**
Paragangliomas are neuroepithelial cell tumors of the ganglia and a subtype of pheochromocytomas. Mediastinal location is rare and is usually associated with a more complex course due to being highly vascular and adjacent to vital structures. We describe a functional mediastinal paraganglioma discovered during the work up for resistant hypertension during pregnancy.
Case presentation:
A 29-year-old pregnant lady with recently diagnosed gestational hypertension on methyldopa presented with hypertensive crises with a blood pressure of 226/118. She reported headaches and palpitations but denied any chest pain, blurred vision or focal weakness. Her physical examination was unremarkable. Labetolol and hydralazine were started. Work up for secondary hypertension was significant for elevated plasma metanephrine 1.16 nmol/l, normetanephrine > 4.55nmol/l and urine venylmandelic acid 26 mg/g. Subsequent abdominal MRI failed to show any abnormality and the decision of medical management until delivery was taken. Unfortunately, due to fetal bradycardia emergency C-section was performed and the baby was born prematurely at 32 weeks.
Six weeks later, She underwent Metaiodobenzylguanidine (MIBG), which positively localized abnormal activity in left chest. Further cardiac MRI revealed a mass along the left atrial border measuring 3.5x3cm. She underwent surgical resection which showed a mass 3.9x3.8x2.8 cm located in the middle mediastinum adjacent to the right superior pulmonary vein and adherent to the left atrium. Its location was challenging requiring cardiopulmonary bypass for complete resection. Pathology confirmed mediastinal paraganglioma with SDHB mutation (c.574T>C, P.C192R). Blood pressure control was maintained and genetic counseling was planned.
Discussion:
Mediastinal paragangliomas are rare catecholamine secreting tumors accounting for 2% of their incidence. Presentation is usually related to hormone overproduction or compression effect.
To the best of our knowledge, this is one of the few cases of mediastinal paragangliomas discovered during pregnancy.
Familial paragangliomas are usually associated with succinate dehydrogenase (SDH) gene mutation with SDHB carrying the highest malignancy potential 20%.
These tumors are characterized by being resistant to radiation and chemotherapy making surgical resection the treatment of choice. Periodic screening for local recurrence is vital for early detection to prevent significant mortalities and morbidities.

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Nothing to Disclose: MK, MT, TY, FH

Pub # P2-645

Session Information POSTER SESSION: CLINICAL - Adrenal Tumor Cases (1:30 PM-3:30 PM)

Title Clinical Outcome Thirty-Five Years after Unilateral Adrenalectomy in a Patient with Idiopathic Hyperaldosteronism and Re-Evaluation of the Resected Gland by Immunohistochemical Staining

Author String K Nomura, H Saito, T Nishikawa, M Aiba
Tokyo Women's Medical University, Tokyo, Japan; Institute of Clinical Endocrinology, Tokyo Women's Medical University, Tokyo, Japan; Tokyo Women's Medical University, Tokyo, Japan; Tokyo Women's Medical University Medical Center East, Tokyo, Japan

Body *Purpose* Idiopathic hyperaldosteronism (IHA) has typically been treated medically. Recent studies, however, have reported the effectiveness of unilateral adrenalectomy (UAdx) in relieving symptoms and correcting hypertension. The present report shows the long-term outcome of a patient with IHA whose right adrenal gland was resected 35 years ago. Specimens from the resected gland were re-examined using modern immunohistochemical analysis. *Material and Methods* The surgically resected adrenal gland was maintained in formalin-fixed paraffin embedded slices for 35 years. These were stained immunohistochemically using specific antibodies against P450aldo, P45011beta, and 3betaHSD. The patient's plasma aldosterone concentration (PAC) and plasma renin activity (PRA) were measured in the supine position. *Medical records* The patient is an 80-year-old male. He was hypertensive since age 33, and was examined upon admission in 1976 at age 44. His data were: 171 cm height, 86 kg, 198/130 mmHg, urine protein 1+, sugar 3+, creatinine 1.2 mg/dl, Na/K 146/3.3 mEq/l. PRA (0.8 ng/ml/h) did not increase after 2 hours of standing and a bolus iv infusion of 80 mg furosemide. PAC was not suppressed (189 pg/ml) by 20 g salt intake for 3 days. CT scanning was not available at this time. Examinations by venography, retroperitoneal pneumography, and scintigraphy suggested the presence of an adenoma on the right gland. The right gland was surgically resected and was not found to contain any adenoma. He was diagnosed with IHA. During the 35 years since UAdx, he was diagnosed with transitional cell carcinoma of the bladder in 1991 and early gastric carcinoma in 2009. He is now being treated for hypertension, diabetes, and dyslipidemia. His present data are as follows: Na/K 138/4.5, creatinine 1.94, urine protein 2+, PAC 113, and PRA 1.3. Although his renal function worsened, he has now lived longer than the average Japanese man (79.59 years) with good ADL. Adrenal CT scans in 2002 and 2010 showed hyperplasia of the left gland without any nodules. The surgical specimens were re-examined. The hyperplastic zona glomerulosa cells contained spironolactone bodies, and showed positive staining for P450aldo and 3beta HSD and were negative for P45011beta. *Conclusion* The present case demonstrates a good long-term prognosis for IHA after UAdx. It also shows that older specimens can be pathologically re-evaluated by modern immunohistochemical analysis.

Nothing to Disclose: KN, HS, TN, MA

Pub # P2-646

Session Information POSTER SESSION: CLINICAL - Adrenal Tumor Cases (1:30 PM-3:30 PM)

Title Challenges with Adrenal Venous Sampling for Primary Aldosteronism

Author String G Smushkin, WF Young, Jr
Mayo Clinic, Rochester, MN

Body The diagnosis of primary aldosteronism (PA) is relatively straightforward. However, distinguishing unilateral adrenal disease from bilateral idiopathic hyperaldosteronism (IHA) can be challenging. CT findings are often misleading because a significant proportion of patients with a unilateral adrenal mass prove to have IHA. Moreover, imaging may reveal bilateral micronodular abnormalities, which does not necessarily imply bilateral hyperplasia. Similarly, the absence of a mass does not exclude an aldosterone-producing adenoma (APA), as many APAs are very small (<5 mm). To highlight some of the challenges in the PA subtype evaluation, we report 3 consecutive cases seen at Mayo Clinic during 1 week in 2010.

1. Unilateral APA in a patient with bilateral adrenal nodules on CT: 39-yr-old woman with an 8 yr history of marked hypokalemia and hypertension. Evaluation at an outside institution included: confirmation of PA; bilateral adrenal nodules on CT; unsuccessful adrenal venous sampling (AVS). AVS at Mayo Clinic showed left-sided aldosterone excess with a cortisol-corrected aldosterone lateralization ratio of 22.3:1. Following left adrenalectomy, plasma aldosterone concentration was undetectable.
2. Bilateral IHA in a patient with a unilateral adrenal mass on CT: 53-yr-old man with a 12 yr history of mild hypokalemia and poorly controlled hypertension. Evaluation at an outside institution included: confirmation of PA; 1.8 cm adrenal left adrenal mass on CT. AVS at Mayo Clinic showed bilateral aldosterone excess. Medical treatment with eplerenone was initiated.
3. Unilateral APA in a patient with 3 prior unsuccessful attempts at AVS: 60-yr-old man with a 20-year history of poorly controlled hypertension and spontaneous hypokalemia. Evaluation at an outside institution included: confirmation of PA; 1.3 cm right adrenal mass on CT; unsuccessful adrenal AVS on 3 separate attempts. AVS at Mayo Clinic showed right-sided aldosterone excess with a cortisol-corrected aldosterone lateralization ratio of 26.2:1. Following right adrenalectomy, plasma aldosterone concentration was undetectable.

Conclusion: AVS is an essential step in the evaluation of PA and should be considered in patients who want to pursue the surgical option. When the subtype evaluation is guided by imaging alone, some patients have noncurative surgery and others, who could be surgically cured, will not be offered the surgical option. Access to an experienced AVS program is key to optimal management of PA.

Nothing to Disclose: GS, WFY

Pub # P2-647

Session Information POSTER SESSION: CLINICAL - Adrenal Tumor Cases (1:30 PM-3:30 PM)

Title Usefulness of the ACTH Administration Test in Three Cases with Hypercortisolism and Hyperaldosteronism

Author String D Kukidome, K Koga, M Igata, J Kawashima, S Shimoda, Y Shigematu, K Nishi, Y Yamashita, M Eto, H Sasano, N Miyamura, E Araki
Honjo, Kumamoto, Japan; Honjo, Kumamoto, Japan; Honjo, Kumamoto, Japan; Seiryomachi, Aobaku, Sendai, Japan

Body Comorbidity of two adrenal disorders in a patient, hyperaldosteronism (HA) and hypercortisolism (HC), should not be overlooked to select an optimal treatment strategy for the patient, and HA in such cases tends to be masked by low ACTH levels induced by HC. We report herein three cases in which the ACTH administration test was useful to find the complications.

Case 1: A 36-y.o. female with Cushingoid features, having two separate tumors in left adrenal, was admitted. Standard examinations indicated overt Cushing syndrome (CS), however, we suspected for coinciding HA in the patient, because aldosterone (A) to cortisol (C) ratio after ACTH stimulation (AsACR) was elevated to 1.2 ([ng/dl]/[[mu]g/dl]). Aldosterone to renin ratio (ARR) was not elevated (=16.3 [ng/dl]/[ng/ml/hr]). Adrenal vein sampling with ACTH stimulation (Ac-AVS) revealed high concentration of both A and C only in left adrenal vein (AV), and postsurgical histopathological analysis indicated that A was produced by only one tumor and C was by the other, mainly.

Case 2: A 63-y.o. male was referred with suspicion of primary aldosteronism (PA) because of severe hypertension and hypokalemia with no Cushingoid features, also having two separate tumors in left adrenal. ARR (=62.5) and the captopril challenge test were consistent with PA, but autonomous secretion of C was also confirmed, being diagnosed as having subclinical Cushing syndrome (SCS). AsACR was elevated to 1.65, Ac-AVS revealed high concentration of both A and C in left AV. The patient underwent left adrenalectomy and the symptoms have been ameliorated. Histopathological analysis revealed the same results as Case 1

Case 3: A 42-y.o. female with typical Cushingoid feature and single tumor in left adrenal was analyzed. CS was the diagnosis; however, coinciding HA was suspected because AsACR was 0.64. While being lower than those in Case 1 and 2, the value was at the average level observed in patients with CS or SCS (0.07-1.65) in our institution. Ac-AVS was performed revealing high concentrations of C only in left AV, and of A only in right AV. Based on these results, partial left adrenalectomy was undergone, preserving normal left adrenal gland to be prepared for possible right total adrenalectomy in the future. Pathological analysis was compatible with C-producing adenoma, and plasma C levels sharply decreased after operation. The patient is now under careful observation for exacerbation of HA accompanying with ACTH recovery.

Nothing to Disclose: DK, KK, MI, JK, SS, YS, KN, YY, ME, HS, NM, EA

Pub # P2-648

Session Information POSTER SESSION: CLINICAL - Adrenal Tumor Cases (1:30 PM-3:30 PM)

Title Hypertension and Hypokalemia: A Missed Diagnosis of Primary Aldosteronism

Author String N Vaishnav, LM Eck
University of Kansas, Kansas City, KS

Body

Introduction: Primary aldosteronism (PA) is the excess production of aldosterone which results in potassium wasting and sodium retention; individuals with this condition present with difficult to control hypertension and often, hypokalemia.

Case Description: A 62 year-old male with a long standing history of difficult to control hypertension and profound hypokalemia was referred for diabetes management. Given his notable hypertensive history, an aldosterone to plasma renin activity (PRA) ratio was measured and was suggestive of PA. This was confirmed with an elevated 24-hour urine aldosterone after oral salt loading. Adrenal computed tomography (CT) revealed a 1 cm nodule in the left adrenal gland. Adrenal vein sampling (AVS) was pursued as the patient was older than 40 years and desired a surgical cure; this revealed a significantly elevated left to right adrenal cortisol corrected aldosterone ratio. A left adrenalectomy was done with subsequent downward titration of anti-hypertensive agents and discontinuation of all potassium supplementation.

Discussion: PA was first reported in 1954. Since that time, multiple subtypes have been described, the most common being bilateral adrenal hyperplasia (BAH) followed by aldosterone-producing adenoma (APA). Although PA is not common, it is under-diagnosed. Screening involves measuring the aldosterone to PRA ratio in those with hypertension and hypokalemia, severe hypertension, resistant hypertension, hypertension with an adrenal [ldquo]incidentaloma,[rdquo] hypertension with an early onset hypertensive family history, and hypertensive first-degree relatives of patients with PA. PA is suspected when the PRA is low and aldosterone is high. To confirm, sodium loaded 24-hour urine aldosterone is measured; physiologically, a sodium load should suppress aldosterone. Once PA is confirmed, adrenal CT is pursued. AVS is required for: 1) bilateral lesions to distinguish an APA vs. BAH, 2) unilateral lesion if age is > 40 years 3) confirmation of PA prior to surgical removal, and 4) imaging that did not reveal any lesions. Curative surgical intervention can be pursued if an APA is identified.

Conclusion: Several situations warrant workup for hypertension secondary to PA. This case illustrates the most common presenting signs of PA, uncontrolled hypertension and unexplainable hypokalemia. Early recognition can lead to curative surgery and decreased morbidity related to longstanding, uncontrolled hypertension.

Nothing to Disclose: NV, LME

Pub # P2-649

Session Information POSTER SESSION: CLINICAL - Adrenal Tumor Cases (1:30 PM-3:30 PM)

Title Co-Existence of Cortisol-Secreting Adenoma with Primary Aldosteronism Persisting after Unilateral Adrenalectomy

Author String J Ahlan, LM Mermejo, S Grunenwald, A Lacroix
Centre Hospitalier de l'Université de Montréal (CHUM), Montréal, Canada

Body
Background: Co-secretion of cortisol and aldosterone from a single adrenal adenoma have been reported. Rarer are the cases of simultaneous distinct adenomas secreting independently either cortisol or aldosterone. We now report a case of co-secretion of cortisol and aldosterone from unilateral adenoma under regulation of aberrant hormone receptors, but in whom primary aldosteronism persisted after unilateral adrenalectomy.
Clinical case : A 41-year-old Haitian woman with premature coronary artery disease and hypertension underwent investigation for pancreatitis. Abdominal CT uncovered a 3.7x2.7 cm left hypodense adrenal lesion, while the right adrenal was normal. The patient noted mild hirsutism and weight gain of 5 kg in the last 2 years. Blood pressure control required 3 antihypertensive drugs and BMI was 30.2 kg/m². Initial testing revealed normal 24-hr urinary catecholamines, elevated plasma cortisol (F) of 615 nmol/L following 1-mg overnight dexamethasone (N<50), UFC of 403 nmol/d (N<330), suppressed ACTH, plasma aldosterone/renin ratio (ARR) of 1332 (N<555) and 24-hour urinary aldosterone at 1235 nmol/d (N<67) with normal serum potassium levels.
Investigation: *In vivo* screening for aberrant hormone receptors identified the following ACTH and renin-independent maximal increases over baseline: 2h upright posture (F:31%, A:237%), 10 IU vasopressin im (F:85%, A:40%), tegaserod (F:17%, A:605%), LHRH 100 [micro]g iv (F:39%, A:42%); there were no F or A responses to mixed meal, TRH, desmopressin, glucagon, or LH tests. A left adrenalectomy was performed and a benign 19 g adenoma without zona glomerulosa nodules was diagnosed. Post-operatively, ACTH and cortisol were suppressed and she required hydrocortisone replacement for 18 months. However, ARR and urinary aldosterone remained elevated (>1500 and 113 nmol/d respectively), with residual hypertension. There was no aberrant increase in F or A to upright posture test with persistent elevation of ARR and suppressed renin. Therapy with spironolactone provided excellent control of blood pressure and right adrenal is still normal on CT scan performed 6 years after left adrenalectomy.
Conclusion: This patient had a mixed cortisol and aldosterone secreting adenoma regulated by aberrant hormone receptors. Residual primary aldosteronism following removal of this adenoma may be secondary to idiopathic hyperaldosteronism of the right adrenal without aberrant regulation by upright posture.

Nothing to Disclose: JA, LMM, SG, AL

Pub # P2-650

Session Information POSTER SESSION: CLINICAL - Adrenal Tumor Cases (1:30 PM-3:30 PM)

Title Successful Treatment of Adrenal Remnant Tissue Using Alcohol Ablation in a Patient with Episodic Cushing Disease

Author String JM Augustine, RL Schaffer, TC Friedman
Charles R Drew University of Medicine and Science, Los Angeles, CA; Scripps Clinic and Green Hospital, La Jolla, CA

Body

Background: The first line of treatment for Cushing's disease is transphenoidal resection of the pituitary tumor with the second line of treatment either bilateral adrenalectomy or radiosurgery. Recurrence of hypercortisolism rarely occurs following bilateral adrenalectomy and is due to adrenal remnant tissue either in the adrenal bed or adrenal rest tissue in an ectopic location and is usually diagnosed by a measurable morning plasma cortisol with the patient on dexamethasone. To our knowledge, this is the first report of successful treatment of adrenal remnant tissue with the minimally invasive use of ethanol ablation.

Case Report: A 25-year-old woman presented with persistent signs and symptoms of Cushing's syndrome, including obesity (BMI 33.3), violaceous striae, hypertriglyceridemia, fatigue, sleep disturbances, and hypertension in spite of 2 unsuccessful transphenoidal resections of a pituitary microadenoma, as well as a laparoscopic bilateral adrenalectomy in 2007. While off of hydrocortisone replacement, the patient had undetectable morning serum cortisol levels and multiple positive 0000 h salivary cortisols of 0.78, 0.54 and 0.30 mcg/dL (nl for 0000 h; < 0.01-0.090 ug/dL) and on dexamethasone (0.25 mg/day) replacement, she had a detectable urinary free cortisol of 2.4 mcg/24 hr (4-50 mg/24 hr). CT imaging showed a soft tissue area of uncertain etiology in the left adrenal fossa measuring 11 mm x 5 mm. The right adrenal bed was clear of soft tissue and there were no other findings suspicious of adrenal rest tissue. Because of uncertainty as to whether the tissue in the left adrenal fossa was indeed adrenal remnant tissue, we performed a lower-risk, CT-guided ethanol ablation rather than an open re-operative surgery.

After one-year, the patient experienced dramatic clinical improvement and no longer appears cushingoid. The patient has lost 40 pounds and now has a BMI of 26.6, her lipids and blood pressure have normalized, and her striae are no longer purple in color. Her latest labs show that her cortisol levels are undetectable, and the patient is now on a physiologic dose of replacement hydrocortisone at 17.5 mg/day.

Conclusion: Adrenal remnant tissue may present with night-time hypercortisolism that leads to signs and symptoms of Cushing's syndrome and may be episodic. We believe this to be the first reported successful alcohol ablation of adrenal remnant tissue in a patient with refractory Cushing's syndrome despite conventional treatments.

Nothing to Disclose: JMA, RLS, TCF

Pub # P2-651

Session Information POSTER SESSION: CLINICAL - Adrenal Tumor Cases (1:30 PM-3:30 PM)

Title Atypical Presentation of Cushing Syndrome (CS) Secondary to Ectopic Adrenocorticotropic (ACTH) Secretion in an Elderly Patient

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Body

Introduction
We present a case of Cushing's syndrome secondary to ectopic ACTH secretion in an elderly female admitted with weight loss, myopathy, rapidly progressive dementia, new onset diabetes, and hypertension.

Clinical Case
A 92 year-old female presented with rapidly progressive dementia, myopathy, fatigue and weight loss. On physical exam, she was hypertensive (151/81 mmHg), frail and lethargic. Proximal myopathy, peripheral edema, bruisability and a round face with well-demarcated red ecchymotic patches were noticed. Laboratory analysis showed leukocytosis, metabolic alkalosis, hypokalemia, hyperglycemia and hypercortisolism (cortisol am 62-84 ug/dL, nl 4 - 22 ug/dl). CS was suspected. Additional labs showed markedly elevated 24 h urinary free cortisol level (1548 ug/dl, nl<45 ug/dl) and ACTH (187-277 pg/ml, nl 6-58 pg/ml) consistent with ACTH-dependent CS. A lack of suppression with high dose dexamethasone and negative pituitary MRI confirmed ectopic ACTH secretion (EAS). Cat scan of chest and abdomen showed diffuse bilateral adrenal thickening and liver masses. An ocreotide scan was negative. No primary tumor was identified. Chromogranin A was elevated at 418 ng/ml(nl 0-50ng/ml), serotonin, glucagon and 5-HIAA were normal. Further work up was declined. Treatment with metyrapone was initiated. Cortisol and ACTH levels decreased. Blood pressure and glucose normalized, however the patient expired 2 weeks later.

Discussion
Ectopic secretion of ACTH represents 10 - 15% of CS. EAS can be a diagnostic challenge, presenting with nonspecific symptoms(1,2). In our patient, newly diagnosed diabetes, myopathy, altered mental status, leukocytosis and atypical skin lesions raised suspicion for CS. The most common causes of EAS are neuroendocrine tumors (52.5%)(3). The optimal treatment is resection or destruction of ACTH-secreting neoplasm, which is only possible in a small proportion of patients(4). Medical or surgical adrenalectomy are necessary when the source of ACTH cannot be removed. Ketoconazole and metyrapone can be used to control hypercortisolism in nonsurgical candidates. Our patient was treated with metyrapone, but despite improvement in her cortisol levels she expired.

Conclusion
EAS can manifest with nonspecific symptoms. Therefore, a high index of suspicion is recommended in patients with rapid unexplained onset of psychiatric, neuromuscular or infectious symptoms, especially if they coincide with bruisability, new onset diabetes and hypertension.

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(2) Ioannis I et al., J Clin Endocrinol Metab 2005; 90(8):4955-4962
(3) Aniszewski JP et al., World J surg 2001; 25 (7): 934-940
(4) Andrea M et al., J Clin Endocrinol Metab 2006; 91(2):371-377

Nothing to Disclose: BA, KRR, MN, RP, AG

Pub # P2-652

Session Information POSTER SESSION: CLINICAL - Adrenal Tumor Cases (1:30 PM-3:30 PM)

Title A Case of Preclinical Cushing Syndrome with Dramatic Reduction of Body Weight after Surgical Resection of a Cortisol-Producing Adenoma

Author String K Iida, S Iguchi, Y Hino, T Ohara, T Kurahashi, K Chihara
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Body Background: Preclinical Cushing's syndrome (PCS) is characterized by autonomous secretion of cortisol from the adrenal tumor but without the typical Cushing's syndrome phenotype. Because cortisol secretion by the tumor is mild in PCS, the indication of surgical operation as a treatment for PCS patients remains controversial.

Clinical case: We present the case of a 32-year-old woman who had obesity, hypertension, and recurrent urolithiasis and had been treated with anti-hypertensive drugs. A tumor (diameter, 3 cm) was coincidentally identified in the patient's left adrenal gland by computed tomography, and she was referred to our hospital for further evaluation. Physical examination revealed that her height, weight, and body mass index were 153 cm, 92 kg, and 39.3 kg/m^2 , respectively. She was obese but did not have a Cushingoid appearance. Endocrinological examination revealed that the morning cortisol concentration was $15.8 \text{ [}\mu\text{g/dl]}$ and adrenocorticotropic hormone was undetectable. There was no circadian rhythm in the serum cortisol levels. The urinary free cortisol level was within the normal range ($56 \text{ [}\mu\text{g/day]}$). An overnight 8-mg dexamethasone suppression test failed to suppress the serum cortisol levels. ^{131}I -adosterol scintigraphy revealed increased accumulation of radioactivity in the adrenal tumor, but no radioactivity in the right adrenal gland. Based on these findings, the patient was diagnosed with PCS. A 75-g oral glucose tolerance test revealed that she had impaired glucose tolerance. Her bone mineral density was 85% of the mean young adult bone mineral density. The left adrenal tumor was surgically resected and benign adenoma was diagnosed histologically. Seven months after the operation, the patient's body weight reduced dramatically to 70 kg and her blood pressure normalized without medication.

Conclusion: This case highlights the importance of considering surgical treatment in PCS patients with obesity, hypertension, diabetes mellitus, or osteoporosis.

Nothing to Disclose: KI, SI, YH, TO, TK, KC

Pub # P2-653

Session Information POSTER SESSION: CLINICAL - Adrenal Tumor Cases (1:30 PM-3:30 PM)

Title Perforated Diverticulum in a Patient with Cushing Disease

Author String A de Havenon, J Ehrenkrantz
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Body

Introduction
Cushing's syndrome is associated with gastritis, gastroduodenal ulcers, and gastric perforation.(1) Lower gastrointestinal complications in patients with Cushing's disease, however, have not been reported.(2)
Case presentation
A 71-year-old female with a 10-year history of hypertension, hypokalemia, and diabetes mellitus presented with abdominal pain and distension. On admission the patient had a BP of 202/97 mm and a K⁺ level of 2.1 mEq/L. Her physical exam was significant for a moon face, dorsocervical fat pad, central obesity, proximal myopathy, and absent bowel sounds. Abdominal CT revealed dilated loops of the distal small bowel, diverticulosis, and bilateral adrenal gland enlargement. The patient received i.v. KCl and her abdominal pain and ileus resolved.
Following the administration of 8 mg dexamethaxone at 12 AM, the 8 AM cortisol was 94 mcg/dL and ACTH 100 pg/mL. An MRI of the brain identified a focal decrease in contrast uptake in the right anterior pituitary gland. Bilateral inferior petrosal sinus sampling showed a post-CRH petrosal to peripheral ratio of 7.7 on the right and 5.1 on the left. The patient underwent transphenoidal hypophysectomy but continued to have elevated cortisol and ACTH levels.
On the 12th postoperative day the patient presented with sepsis. Laparotomy revealed an intra-abdominal abscess and extensive diverticulitis with perforation. She refused aggressive therapy and died of post-operative complications. Pertinent autopsy findings consisted of multiple intra-abdominal, pelvic and retroperitoneal abscesses, colonic mesenteric atrophy, and numerous thin-walled diverticula, one of which was perforated. Examination of the parasellar region identified residual ACTH-secreting cells consistent with an adenoma attached to the superior aspect of the pituitary stalk.

Discussion
This case illustrates the potential for fulminant diverticular disease complications in Cushing's disease. Possible pathophysiological mechanisms which may exacerbate diverticular disease in patients with hypercortisolism include inhibition of epidermal cell division, decreased collagen synthesis, decreased GI motility, impaired cellular immune function, and blunting of the acute inflammatory response.(3, 4) These factors can contribute to the increased risk of diverticular perforation and abscess formation in patients with Cushing's disease.

(1) Ellershaw JE, Kelly MJ. Glucocorticoids and peptic ulceration. *Palliat Med.* 1994 Oct;8(4):313-9.
(2) Mpofu S, et al. Steroids, non-steroidal anti-inflammatory drugs, and sigmoid diverticular abscess perforation in rheumatic conditions. *Ann Rheum Dis.* 2004 May;63(5):588-90.
(3) Sartorio A, et al. Evaluation of markers of bone and collagen turnover in patients with active and preclinical Cushing's syndrome and in patients with adrenal incidentaloma. *Eur J Endocrinol.* 1998 Feb;138(2):146-52.
(4) Jabbour SA. Cutaneous manifestations of endocrine disorders: a guide for dermatologists. *Am J Clin Dermatol.* 2003;4(5):315-31.

Nothing to Disclose: AdH, JE

Pub # P2-654

Session Information POSTER SESSION: CLINICAL - Adrenal Tumor Cases (1:30 PM-3:30 PM)

Title Expression of Vasopressin Receptors in Familial Cushing Syndrome Caused by Vasopressin-Sensitive ACTH-Independent Macronodular Adrenal Hyperplasia

Author String S-H Lee, S Moon, S-H Ko, K-H Baek, S-A Chang, H-M Cho, Y-B Ahn, K-W Lee
The Catholic University of Korea, Suwon, Republic of Korea; The Catholic University of Korea, Suwon, Republic of Korea

Body Background: ACTH-independent macronodular adrenal hyperplasia (AIMAH) is a very rare cause of Cushing's syndrome. Hypersecretion of cortisol regulated by ectopic expression or eutopic overexpression of several G-protein-coupled receptors have been demonstrated in these patients. We present a family with four members affected by vasopressin-sensitive AIMAH, with in vivo and in vitro studies.
Clinical case: The proband was a 44-year-old female (III-4) with central obesity, easy bruising, amenorrhea and hypertension. Initial tests showed elevated basal serum cortisol (30.6 ug/dL), elevated 24hr urine free cortisol (697 ug/day) and suppressed plasma ACTH level (not detected). Cortisol secretions were not suppressed by either a low or a high-dose dexamethasone suppression tests. Bilateral adrenal macronodular enlargement was seen on abdominal CT scan. In vivo screening tests with various stimulations showed 289% increase in cortisol secretion in response to vasopressin. The patient's 48-year-old sister (III-2) revealed similar findings with 358% increase in cortisol secretion in response to vasopressin. The two sisters had laparoscopic bilateral adrenalectomy and the adrenal glands weighed 60 and 73 g, respectively. Reviewing the medical records, it was found that the patient's mother and her sister also had been diagnosed as vasopressin-sensitive AIMAH.
In vitro studies: The relative amount of vasopressin receptor (V1aR, V1bR and V2R) mRNAs in adrenal tissues were compared with normal adrenal tissue or normal controls by RT-PCR. The expression of V1aR was increased 1.3-fold in III-4 and 2-fold in III-2. Ectopic expression of V1bR and V2R were noted in both patients. Immunohistochemical stainings for V1aR were compatible with RT-PCR results. V2R were positive in both patients while V1bR were positive in only III-4.
Conclusion: In this family, we have shown that vasopressin sensitivity caused AIMAH with relevant in vitro data on vasopressin receptor expression.

Nothing to Disclose: S-HL, SM, S-HK, K-HB, S-AC, H-MC, Y-BA, K-WL

Pub # P2-655

Session Information POSTER SESSION: CLINICAL - Adrenal Tumor Cases (1:30 PM-3:30 PM)

Title Bilateral Macronodular Adrenal Hyperplasia Co-Secreting Aldosterone and Cortisol under the Regulation of Aberrant Hormone Receptors: Clinical Response to β -Blockers

Author String E D'Aoust, S Grunenwald, LMM Mermejo, I Bourdeau, A Lacroix
Centre Hospitalier de l'Université de Montréal (CHUM), Montreal, Canada

Body
Background: ACTH-independent bilateral macronodular adrenal hyperplasia (AIMAH) usually presents with sub-clinical Cushing's syndrome (CS) or overt CS. Rarely there is co-secretion of cortisol (F) and aldosterone (A) or sex steroids. AIMAH is of heterogeneous etiology, but is frequently regulated by diverse aberrant hormone receptors. We report a patient presenting as primary aldosteronism in which AIMAH oversecreted A and F in association with non-secreting pancreatic neuroendocrine tumor (NET).
Case report: A 36-yr old Haitian male was evaluated for high blood pressure requiring 3 antihypertensive drugs. Plasma A/renin ratios and 24-h urinary A were elevated. Adrenal CT scan revealed bilateral macronodular adrenals (R: 7.5, L: 8.9 cm) and a 2.8 cm calcified pancreatic nodule. There were no overt CS signs, but F was 495 nmol/L post 1-mg overnight dexamethasone (N:<50), basal ACTH was 0.5 pmole/L (N: 2-11), and 24h UFC ranged from 333 to 1103 nmol/d (N:<330). Serum fasting glucose PTH and gastrin were normal without diarrhea or flushing. Tests examining several potential aberrant adrenal receptors were performed. ACTH and renin-independent responses were found with 2h-upright posture (F increase: 138%; A 162%), 10 IU vasopressin i.m. (F: 183%; A: 133%) and isoproterenol infusion (F:73%; A:53%). No abnormal F or A responses followed mixed meal, TRH, LHRH, metoclopramide or desmopressin tests. As β -blocker alone (no V1AVP receptor antagonist available) could not normalise steroid secretion and pancreatic surgery was required, a simultaneous left adrenalectomy was performed; benign pancreatic NET and AIMAH were found at pathology. Post operatively, UFC normalised initially, but increased between 345-535 nmol/L and urinary A to 114 nmol/d (N<67). Nadolol (to 80 mg/d) was added to amiloride 10 mg/d; 24-h UFC and urinary A levels normalised during 24 months follow-up with normal blood pressure and weight loss of 13 kg. His father also has hypertension and AIMAH, but declined further investigation; no *MEN1* mutation was found in this patient.
Conclusion: This case presented AIMAH with primary aldosteronism combined with cortisol secretion which persisted after unilateral adrenalectomy; identification of aberrant regulation by vasopressin and catecholamines allowed normalisation of excess steroidogenesis with β -blockers.
Coexistence of pancreatic neuroendocrine tumor and familial AIMAH suggests a new genetic etiology as no *MEN1* mutation was found.

Nothing to Disclose: ED, SG, LMMM, IB, AL

Pub # P2-656

Session Information POSTER SESSION: CLINICAL - Adrenal Tumor Cases (1:30 PM-3:30 PM)

Title Bilateral Macronodular Adrenal Hyperplasia -- A Rare Cause of Cushing Syndrome Presenting with Hypogonadotropic Hypogonadism

Author String A Srinath, V Winstanley, JD Newell-Price, A Lulsegged
Princess Royal University Hospital, Orpington, UK; University of Sheffield, Sheffield, UK

Body Background: ACTH Independent Macronodular Adrenal Hyperplasia (AIMAH) is a rare cause of Cushing syndrome and may present in a variety of ways.
Case: A 31y male was referred with a 3-4 year history of loss of libido and erectile dysfunction. Investigation: revealed, hypogonadotrophic hypogonadism (HH); total testosterone 4.2nmol/l (8.4-28.7), LH 2.1 iu/l (1.9-12.5), FSH 2.0 iu/l (2.5-10.2). He was also noted to be obese and had wide abdominal striae. Clinical suspicion of Cushing's syndrome (CS) was confirmed with raised 24-hour urinary free cortisol 3325nmol (0-460), serum cortisol 611nmol/L after overnight dexamethasone suppression (normal <50), and 0900h plasma ACTH 5ng/L (10-56). MRI adrenals showed large bi-lateral macronodular hyperplasia. DEXA showed osteoporosis of spine (T score -2.5). Low dose metyrapone 250mg BID was commenced with rapid reduction in urinary cortisol excretion to 188nmol/24 hours (0 - 460nmol/24h). Definitive treatment was then undertaken via bilateral laparoscopic adrenalectomy. Post-op cortisol was 7nmol/L indicating cure and he was discharged on hydrocortisone and fludrocortisone.
Learning points:
(1) Recognition of Cushings as a cause of HH. It might have been easy to overlook CS and ascribe a lot of his features (including osteoporosis) to HH alone. HH is reversible with successful treatment of CS.
(2) AIMAH is a rare cause of Cushings syndrome (<1% cases). Affected adrenals can measure up to 13cm. Clinical manifestations can be mild and develop over a longer time compared to other causes of CS. Treatment options include removal of one (largest) adrenal, bilateral adrenalectomies or long term medical treatment. Unilateral adrenalectomies maybe considered in mild cases with appropriate follow up but this increases the risk of uncontrolled disease. Medical therapy likewise may not fully control disease, but small doses of steroidogenesis inhibitors may have a rapid effect, as seen here.
(3) Aberrant adrenocortical hormone receptors which have trophic and steroidogenic stimulatory effects on the adrenal gland by ligands other than ACTH are more common in AIMAH. Here, this was not assessed, but in some AIMAH patients this may help determine the control of secretion of cortisol.
Summary
We emphasise the importance of remembering that CS is an important cause of HH, and that AIMAH may be highly sensitive to low doses of steroidogenesis inhibitors and that care is needed when commencing therapy.
Nothing to Disclose: AS, VW, JDN-P, AL

Pub # P2-657

Session Information POSTER SESSION: CLINICAL - Adrenal Tumor Cases (1:30 PM-3:30 PM)

Title Rapidly Developing Iatrogenic Cushing Syndrome after Short-Term Topical Application of Combination Clotrimazole/Betamethasone Dipropionate Ointment

Author String J Kim, S Kim, H Bae
Chosun University Hospital, Gwangju, Republic of Korea

Body Backgrounds: The combination of clotrimazole/betamethasone dipropionate(CBD) is a mix of an azole antifungal and a high potency glucocorticoid. Clotrimazole is inhibitor of CYP3A4 and can inhibit the metabolism of betamethasone. These drugs interaction may result in a decreased clearance of betamethasone and consequently in an increased risk for iatrogenic Cushing's syndrome. Our patient experienced unusual rapidly developing iatrogenic Cushing's syndrome with secondary adrenal insufficiency after short-term topical of combination CBD ointment application.
Clinical Case: A 26-year-old woman presented with rapidly excessive weight gain. She was prescribed combination clotrimazole/betamethasone dipropionate ointment for her urticaria five weeks before admission. She used small amount on the upper trunk at that time. After 5 days, she applied half of the tube (10g/tube) on her whole body. Since then, she developed weight gain of 30 kg. She did not have any significant past medical history or family history. She had never taken other systemic glucocorticoids and any medications. Physical examination revealed truncal obesity with buffalo hump, round-faced hirsutism, and red striae. The initial laboratory data revealed as follows: WBC 11,430/uL, Hb 13.5 g/dL, platelet count 200,000/uL, serum glucose 99 mg/dL, blood urea nitrogen 24.9 mg/dL, creatinin 1.18 mg/dL, AST 28.8 U/L (normal, 5-40 U/L), ALT 62.9 U/L (normal, 5-40 U/L), sodium 145 mEq/L (normal, 136-146 mEq/L), potassium 4.1 mEq/L (normal, 3.5-5.0 mEq/L), and chloride 100 mEq/L (normal, 98-110 mEq/L). The thyroid function tests disclosed normal ranges. Serum morning cortisol and ACTH at 8.00 AM were < 1 mg/dL and 1.5 pg/mL. We suspected the suppression of hypothalamic-pituitary-adrenal(HPA) axis and performed ACTH stimulation test. Cortisol levels were an inadequate response to stimulation as follows: < 1 mg/dL, 3.9 mg/dL, and 6.1 mg/dL. Thus, we made a diagnosis of iatrogenic Cushing's syndrome with secondary adrenal insufficiency. Conclusion: This is the first case of developing iatrogenic Cushing's syndrome after short-term topical of combination CBD ointment application. Interindividual variation in CYP3A4 activity, in glucocorticoid receptor sensitivity or in glucocorticoid receptor polymorphisms, and drugs interaction are suggested as the underlying mechanisms.

Henk R et al., J Clin Endocrinol Metab 2005; 90:5804

Nothing to Disclose: JK, SK, HB

Pub # P2-658

Session Information POSTER SESSION: CLINICAL - Adrenal Tumor Cases (1:30 PM-3:30 PM)

Title Dehydroepiandrosterone Sulfate (DHEA-S)-Secreting Adrenal Adenoma in a 15-Year-Old Female -- A Case Report

Author String SR Chandratre, F Ugrasbul
Children's Mercy Hospital, University of Missouri at Kansas City, Kansas City, MO

Body
Background: Adrenocortical(AC) tumors are very rare in childhood accounting for less than 0.2% of all pediatric cancers.In United States,only 25 new cases are expected to occur annually,for an estimated annual incidence of 0.2 to 0.3 cases per million in pediatric population.
Clinical Case: 15 year-old otherwise healthy female presented with 6 months history of menstrual irregularities,worsening of facial acne,gradual increase in hirsutism with adult male distribution of facial and body hair,male pattern baldness,deepening of her voice and clitoromegaly on physical examination all suggestive of virilisation.Her blood pressure was not elevated.Hormonal evaluation indicated increased levels of DHEA-S:1456 mcg/dl-(reference range:30 to 550 mcg/dl)and testosterone:142 ng/dl(reference range:5 to 40 ng/dl).Cortisol was not elevated.An abdominal computed tomographic scan detected a right adrenal mass.She had no evidence of metastatic disease(bone scan,computed tomographic scan of neck and chest-normal).A laparoscopic right adrenalectomy was performed and an encapsulated mass measuring 6.5 x 5.5 x 3.5 cm weighing 55 grams was resected.Pathologic diagnosis was right adrenal adenoma.The mitotic rate was low-1/20 per high power field.No areas of necrosis or capsular or vascular invasion noted.Cytogenetic report showed structural and numerical chromosome abnormalities resulting in loss of material from chromosomes 1p,14,16,18 and 21;and gain of chromosomes 2,5,7,9,10,13,19,20 and 22.The levels of DHEA-S and testosterone normalized one week after surgery.She resumed her periods within 1 month of surgical resection of the adenoma.The P53 gene analysis was sent to look for Li-Fraumeni syndrome.
Conclusions: Based on our literature review,a DHEA-S level of above 600 micro-gm/dl is highly suspicious of AC tumor.Our case of AC adenoma was unique with respect to higher number of chromosome abnormalities-a feature of AC carcinoma;and more gain of chromosomes which is usually a feature of a large AC tumor.In the literature,there is no clear genetic or histological distinction between AC adenoma and carcinoma.Hence radical excision with en bloc resection of any local invasion is the best treatment approach for AC tumors.

Nothing to Disclose: SRC, FU

Pub # P2-659

Session Information POSTER SESSION: CLINICAL - Adrenal Tumor Cases (1:30 PM-3:30 PM)

Title Adrenal Sarcoma in Pallister Hall Syndrome

Author String N Shah, M Ruppe
University of Texas at Houston, Houston, TX

Body

Background:
Pallister Hall Syndrome (PHS) is a rare genetic disorder characterized by a wide range of anomalies including central polydactyly, pituitary dysfunction, bifid epiglottis, and hypothalamic hamartomas. Adrenal sarcoma has not been previously described in patients with PHS.

Clinical Case:
A 42 y/o male presented with complaints of nausea, vomiting, and severe abdominal pain. At birth, he was found to have an imperforate anus, polydactyly, and Hirschsprung's disease. After a childhood seizure, a hypothalamic hamartoma was identified and resected, and he subsequently developed panhypopituitarism. He was eventually diagnosed with PHS at age 24. On exam, he had microcephaly, short stature, retained deciduous teeth, bifid epiglottis, small optic discs, and fused toes. He was on hormonal replacement including desmopressin, hydrocortisone, and levothyroxine. CT showed a 8.8 x 7.3 x 8.1 cm right adrenal mass. Low Hounsfield unit attenuation without post contrast enhancement suggested indeterminate adenoma. No evidence of adrenal hormone excess was observed: aldosterone 12.3 ng/dL (normal <16 ng/dL), plasma free metanephrine <10 pg/mL (normal <65 pg/mL), plasma free normetanephrine 116 pg/mL (normal <146 pg/mL), 24hr urinary VMA 5.6 mg/24hr (normal <6.0 mg/24hr), 24hr urinary metanephrine 550 mcg/24hr (normal 182-739 mcg/24hr), 24hr urine normetanephrine 484 mcg/24hr (normal 88-649 mcg/24hr), 24hr urine norepinephrine 63 mcg/24hr (normal 15-100 mcg/24hr), 24hr urinary epinephrine 11 mcg/24hr (normal 2-24 mcg/24hr) and 24hr urinary dopamine 423 mcg/24hr (normal 52-480 mcg/24hr). Patient underwent exploratory laparotomy with en bloc resection of the right adrenal, right hepatectomy, and right radical nephrectomy. Pathology revealed an 11 cm malignant small round blue cell tumor replacing the entire adrenal gland, invading the adrenal capsule with angiolymphatic invasion. Marked increase in mitotic figures was seen (>10/HPF). Immunohistochemical stains were positive for CD99, vimentin, nestin, and chromogranin; and negative for AE1/AE3, desmin, S100 protein, CD3, CD20, CD34, CD45, CD56, myogenin, inhibin, mart-1, and synaptophysin. 60-70% of tumor cells were positive for Ki-67. These findings were consistent with extraskelatal Ewing's sarcoma.

Conclusion:
This is the first known case of adrenal sarcoma in PHS. Surveillance for PHS should include screening for adrenal mass at onset of clinical symptoms.

(1) Biesecker LG et al. Report from the Workshop of Pallister-Hall Syndrome and related phenotypes. Am J Med Genet. 1996; 65: 76-81

(2) Hall JG et al. Congenital hypothalamic hamartoblastoma, hypopituitarism, imperforate anus, and postaxial polydactyly -- a new syndrome? Am J Med Genet. 1980; 7:47-74

Nothing to Disclose: NS, MR

Pub # P2-660

Session Information POSTER SESSION: CLINICAL - Adrenal Tumor Cases (1:30 PM-3:30 PM)

Title How To Worm Out an Unexpected Finding in a Large Adrenal Mass

Author String I Krull, C Lipowsky, W Kolb, M Blobner, M Brandle
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Body **Background:** Echinococcosis is a parasitic infection of humans caused by the larval stage of the tapeworm Echinococcus. The most common species are Echinococcus granulosus and Echinococcus multilocularis, the latter causes alveolar echinococcosis. In Switzerland the incidence of alveolar echinococcosis ranges between 1:250000 to 1:700000 persons per year. Extrahepatic disease is very uncommon (1 percent of cases) and the adrenal gland is an extremely rare site of alveolar echinococcosis affection.

Clinical case: A 43-year-old patient presenting with right abdominal pain and weight loss was admitted to our hospital. The patient's medical history was remarkable of pulmonary sarcoidosis and a calcified liver lesion. Computed tomography and MRI showed a 7x3cm, solid inhomogeneous adrenal mass consisting as well of multiple small cysts and central necrosis. Two calcified liver lesions in segment V and VII as well as lymphadenopathy pericaaval have been documented. The liver lesions seemed unchanged compared with CT scans 13 years ago. The patient revealed no signs or symptoms of an excessive secretion of adrenal hormones. Biochemical evaluation of the adrenal mass yielded a slight increased urinary cortisol excretion (24-h urinary free cortisol 149ug/24h; normal range 13 - 83ug/24h). Further diagnostic testing excluded pheochromocytoma and primary hyperaldosteronism. Due to the size and the characteristics of the adrenal lesion, an adrenocortical carcinoma with autonomous cortisol secretion was suspected. Differential diagnosis included sarcoidosis, tuberculosis, myelolipoma, metastasis and echinococcosis. Surgery was performed and histological examination of the adrenal mass revealed surprisingly echinococcosis multilocularis. Immunodiagnostic tests including Em2-Antigen were also positive. Postoperatively adequate antiparasitic therapy with albendazol 400mg twice daily was started.

Conclusion: This case report provides evidence that echinococcosis alveolaris may present as an adrenal mass. Thus, in the diagnostic workup of an adrenal mass with cystic lesions, differential diagnosis of echinococcosis has to be considered rather than adrenal carcinoma, especially in endemic areas or patients with origin of these areas.

Nothing to Disclose: IK, CL, WK, MB, MB

Pub # P2-661

Session Information POSTER SESSION: CLINICAL - Adrenal Tumor Cases (1:30 PM-3:30 PM)

Title Inhibin B Co-Secretion by a Pediatric Hyperfunctioning Adrenocortical Carcinoma

Author String JRC Zucare, CR Barcelos, AM Faria, MQ Almeida, AM Lerario, FT Denes, GA Alencar, AC Latronico, BB Mendonca, MCBV Fragoso
Hospital das Clinicas, University of São Paulo Medical School, São Paulo, Brazil; Hospital das Clinicas, University of São Paulo Medical School, São Paulo, Brazil; Hospital das Clinicas, University of São Paulo Medical School, São Paulo, Brazil

Body **Background:** Adrenocortical carcinoma (ACC) in pediatric patients is rare. Virilization, alone or in combination with signs of cortisol over-secretion, is the most common endocrine presentation. Inhibin production by adrenocortical tumors is extremely rare, with very few cases reported in adult patients. To our knowledge, this finding has not been previously described in children.
Clinical Case: An 11-month-old female was referred to us for investigation of precocious puberty. Her parents had noticed pubic hair, facial acne, weight gain and increased growth velocity since 7 months of age. At physical examination, her weight was 10.7 Kg (+ 1.57 SD) and the height was 72 cm (- 0.07 SD). She presented clinical signs and symptoms of Cushing's syndrome and virilization, characterized by a plethoric moon face, obesity, acne and Tanner stage II pubic hair, clitoromegaly (3.0 cm), increased axillary sweating and voice deepening. She had a palpable abdominal mass in the right hypochondrium. Hormonal evaluation revealed suppressed ACTH levels (<5 pg/mL), absence of cortisol (12.8 [micro]g/dL) suppression after an overnight 1-mg dexamethasone test, very high testosterone (764 ng/dL, NR:< 14ng/dL), DHEAS (40.000 ng/mL, NR:<1240 ng/mL) and inhibin B levels (1695 pg/mL, NR: 15-90). FSH, LH and estradiol levels were all below the lower limit of detection. Abdominal CT scan showed a 6.5 cm right heterogeneous adrenal mass with no evidence of metastatic lesions. The patient underwent an open right adrenalectomy. Gross pathology identified a 6.5 cm tumor in its greatest diameter of 200 g (stage I according to Sandrini et al.)⁽¹⁾. Histopathological analysis confirmed an ACC (Weiss score 6) and neoplastic cells displayed a cytoplasmic positive staining for inhibin, melan A and synaptophysin. The patient harbors the germline p.R337H TP53 mutation and tumor cells showed evidence of loss of heterozygosity. All postoperative hormone levels fell into the normal range and the patient required glucocorticoid replacement for 3 months. No clinical or biochemical evidence of recurrence has been detected during a ten-month follow-up period. **Conclusion:** This is the first report of a pediatric ACC with co-secretion of androgens, glucocorticoid and inhibin B. We, therefore, suggest that inhibin B should be included in the hormonal evaluation of pediatric patients with adrenocortical tumors.

(1)Sandrini R, Ribeiro RC, DeLacerda L: Childhood adrenocortical tumors. J Clin Endocrinol Metab 82:2027-2031, 1997

Nothing to Disclose: JRCZ, CRB, AMF, MQA, AML, FTD, GAA, ACL, BBM, MCBVF

Pub # P2-662

Session Information POSTER SESSION: CLINICAL - Adrenal Tumor Cases (1:30 PM-3:30 PM)

Title Immunohistochemical Analysis of Steroidogenic Enzyme Expression in Adrenal Cortical Carcinoma Pulmonary Metastases Causing Cushing Syndrome

Author String EF Brutsaert, H Sasano, P Unger, M Beasley, M New, AC Levine
Mount Sinai School of Medicine, New York, NY; Tohoku University, Sendai, Japan

Body Adrenocortical carcinoma (ACC) is a rare cancer with a poor prognosis. We herein present a case of pulmonary metastases from ACC that were secreting fully functional cortisol that produced clinical Cushing's syndrome. We also compared the expression of steroidogenic enzymes in the primary tumor and its late metastases.

Case: A 63 year old postmenopausal female presented with mild symptoms of hyperandrogenism and Cushing's. Laboratory studies revealed ACTH-independent hypercortisolemia as well as elevated androgens (testosterone 163 ng/dl normal 5-51). MRI of the abdomen showed a 4.3 x 3.5 x 2.7 cm left adrenal mass. The patient underwent left adrenalectomy and was diagnosed with a benign adrenal adenoma. Post-operatively, the patient's symptoms resolved and she had normal cortisol levels. Four years later, the patient again developed symptoms of Cushing's but without any signs/symptoms of hyperandrogenism (testosterone was 42 ng/dL normal 6-82). Repeat MRI showed no adrenal masses. However, chest CT showed multiple bilateral lung nodules. Wedge resection of a lung nodule revealed metastases of adrenal origin. The original tumor was reclassified as adrenocortical carcinoma, based on Weiss criteria.

Methods/Results: The original ACC and the pulmonary metastases were stained for the expression of steroidogenic enzymes using standard immunohistochemical techniques. Both primary and metastatic sample stained positively for adrenal 4 binding protein/ steroidogenic factor 1 (Ad4BP/ SF-1) and inhibin- α . In addition, side chain cleavage (CYP11A1), 17 α hydroxylase (CYP17), 3 β hydroxysteroid dehydrogenase (3 β HSD), 21 hydroxylase (CYP21A2) were expressed in the original tumor and in the lung metastases, but showed patterns of disorganized steroidogenesis. Dehydroepiandrosterone-sulfotransferase (DHEA-ST) was present in the original tumor but was not seen in the lung metastases.

Conclusion: This is only the third case in the literature where a late metastasis of an adrenocortical carcinoma produced fully functional steroid hormones causing clinical Cushing's syndrome. Notably, it is the first report that demonstrates immunohistochemical staining for steroidogenic enzyme expression in a primary tumor and its late, hormonally-active metastasis. In spite of disorganized steroidogenesis, these metastases can cause clinical Cushing's syndrome, likely due to a combined effect of excess tumor burden and the high vascularity of these lesions.

Nothing to Disclose: EFB, HS, PU, MB, MN, ACL

Pub # P2-663

Session Information POSTER SESSION: CLINICAL - Adrenal Tumor Cases (1:30 PM-3:30 PM)

Title Efficacy of the Adrenolytic Drug Mitotane in an Aggressive Adrenocortical Carcinoma (ACC) Not Responsive to Conventional Chemotherapy and Experimental Biotherapy

Author String ME Randazzo, E Grossrubatscher, P Dalino Ciaramella, E Giovannini, A Vanzulli, P Loli
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Body ACC is a rare malignancy with a variable but generally poor prognosis; survival is less than 12 months in stage IV patients. Mitotane (MIT) is widely employed in the treatment of advanced ACC and leads to hormone excess control in the majority of patients, objective tumor regression occurring in only about 25%. Isolated case reports of complete and prolonged remission in cases with inoperable or metastatic disease treated by MIT are reported. At least some of the variability in response to MIT may be attributable to subtherapeutic concentrations. A 38 yrs female with progressive metastatic ACC came to our observation on March 2010. At the end of 2008 features of hypercortisolim were observed. CT scan revealed a 78 mm mass in left adrenal, vena cava thrombosis and multiple lung nodules. On march 2009 the patient underwent left nephroadrenalectomy and removal of an intracaval thrombus: the pathological examination confirmed the diagnosis of ACC, immunophenotype: Syn+, CD56+, CgA-, EMA-, CD10-, CK-pool+, Ki67 60%. The patient underwent 3 cycles with combined chemotherapy: cisplatin, etoposide and MIT (taken at the maximum dosage of 1,5 g/die for a short period). Due to rapid progression of disease (lung mets) chemotherapy with taxane and adriamycin was administered from June to Sept 2009, and again stopped after 4 cycles for further progression of lung metastases and appearance of a mesenteric mass. Subsequently she started a new treatment with RAD-001 5 mg and the anti-IGF-1 receptor-human-monoclonal-antibody R1507 16 mg/Kg for 6 cycles until March 2010 with further progression of disease. At the beginning of 2010 Cushing's symptoms re-appeared. Since April 2010 the patient has been taking MIT reaching the maximum dose (6g/die) in 10days, and entering the therapeutic window in 45days, without relevant side effects and rapid regression of Cushingoid features. CT scan of July and Sept 2010, taking as a target the largest lung metastasis and the mesenteric mass, showed a slowing of growth of the first and a stabilization of the second. CT of November 2010 showed size reduction (25-29%) of the majority of lung mets and of the mesenteric mass (16%) and a further slowing of growth of the biggest lung lesion. This case testifies the potential efficacy of MIT in aggressive ACC, when drug blood concentration monitoring ensures the maintenance of a therapeutic range, and demonstrates that drug response can be progressive and must be measured over a medium-term period.

Nothing to Disclose: MER, EG, PDC, EG, AV, PL

Pub # P2-664

Session Information POSTER SESSION: CLINICAL - Adrenal Tumor Cases (1:30 PM-3:30 PM)

Title Identification of an Occult Functioning Cardiac Paraganglioma in a Patient with a Novel SDHD Mutation Causing Familial Paraganglioma Syndrome

Author String JT Kung, DK Oh
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Body **Introduction:** A common feature of familial paraganglioma (PGL) syndromes due to SDHD (succinate dehydrogenase complex subunit D) mutations is head and neck PGLs that are typically biochemically silent (1). We present a case where the identification of an occult source of catecholamine hypersecretion was critical to patient care.

Case: 62 yo female found on exam to have bilateral carotid body PGLs. Pt reported no symptoms of pho spells, but did have HTN and CHF. Pt's brother and a paternal uncle's daughter also had PGLs. Pt's labs revealed increases in plasma normetanephrine (3.39 nmol/L [0-0.89]) as well as urinary norepinephrine (360 mcg/d [0-100]) and normetanephrine (1649 mcg/d [50-650]).

Because biochemical hypersecretion is rare in head and neck PGLs, we undertook an imaging search for other PGLs. MIBG showed mild uptake in the known head and neck PGLs as well as intense uptake in the mediastinum. CT revealed a 4.7-cm cardiac mass between the atria, which is a rare location, as only about 1% of PGLs are intracardiac (1). MIBG uptake does not always correlate with function, but since almost all cardiac PGLs are hyperfunctioning (1), the cardiac mass was identified as the most likely source of excess catecholamines and was excised. Pathology was consistent with PGL. Pt became hypotensive, and by 6 weeks post-op all anti-hypertensives had been discontinued. Repeat 24 hr urine catecholamines and metanephrines were normal, consistent with successful excision of the functioning PGL.

Genetic testing revealed that the pt and her brother share a novel H50D missense mutation in the SDHD gene that is not present in their mother. Their father could not be tested as he died of an MI at age 57, a potential manifestation of catecholamine excess. The pt's family history is consistent with the usual paternal parent-of-origin inheritance pattern of SDHD familial PGL syndromes, in which individuals inheriting mutations from their father are at high risk of developing PGLs, while those inheriting mutations from their mothers are at negligible risk of disease.

Clinical Lessons: Because head and neck PGLs rarely secrete catecholamines, it is important to search for additional PGLs when catecholamine excess is present. Immediately operating on this patient's head and neck PGLs would have missed the actual source of catecholamine excess in her heart, and exposed her to additional surgeries during a risky period of catecholamine hypersecretion.

(1) Erickson D et al., J Clin Endocrinol Metab 2001; 86:5210.

Nothing to Disclose: JTK, DKO

Pub # P2-665

Session Information POSTER SESSION: CLINICAL - Adrenal Tumor Cases (1:30 PM-3:30 PM)

Title Acute Renal Failure, Microangiopathic Hemolysis and Intestinal Ischemia in a 23-Year-Old Woman -- A Puzzling Presentation of Pheochromocytoma

Author String S Sigrist, M Brandle, I Baetscher, I Binet, T Clerici, S Bilz
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Body
Background: Classic symptoms of pheochromocytoma include the triad of episodic headache, sweating and tachycardia and/or arterial hypertension. However, clinical presentation may be atypical.
Clinical case: A 23-year old woman was admitted because of a 3 weeks history of back pain, watery diarrhea a 5 kg weight loss, and intermittent visual disturbances. Arterial hypertension was first noticed 3 years ago and judged as white coat hypertension with no further workup. New onset headaches were noted since 2 years. Personal history was remarkable for hemolytic uremic syndrome (HUS) at age 1 with subsequent recovery. On admission she presented with severe hypertension (220/160 mmHg), spotty hemorrhages in the fundus, tachycardia (120 bpm), renal failure (CrCl 45 ml/min) with low urine output, hematuria with 2-3 % glomerular erythrocytes, proteinuria and microangiopathic hemolysis. Differential diagnosis included glomerulonephritis (GN), HUS and pheochromocytoma. Specific tests for HUS were negative and 24 hour urinary catecholamines were normal. High dose i.v. steroids were started for suspected GN. On day 5, she developed acute abdominal pain with CT findings of intestinal ischemia leading to emergency ileocecal resection. CT also revealed a 2.2 cm left adrenal mass suggestive of a pheochromocytoma. On repeat biochemical testing while on therapy with labetalol plasma free normetanephrine (NM) was in the upper normal range (1.22 nmol/l, n 0.04-1.39) and urinary NM excretion was modestly elevated (7205 nmol/24hr, n <3800). After switching antihypertensive therapy to a calcium channel blocker, further workup including clonidine testing confirmed the diagnosis (plasma free NM 2.67 nmol/l, 14% suppression after 0.3 mg clonidine p.o.) and laparoscopic left adrenalectomy was performed. Family history was unremarkable and screening for mutations in *RET*, *VHL*, *SDHB* and *SDHD* genes was negative. Although arterial hypertension persisted and required still drug therapy, there was no evidence for recurrence of pheochromocytoma during 19 months of follow up.
Conclusions: Pheochromocytoma frequently presents with unusual clinical findings and systemic steroids may precipitate its manifestations by stimulating catecholamine release. The biochemical diagnosis may be difficult and negative testing does not necessarily rule out the diagnosis. Repeat biochemical testing in patients with severe arterial hypertension and a highly suggestive adrenal mass is crucial.

Nothing to Disclose: SS, MB, IB, IB, TC, SB

Pub # P2-666

Session Information POSTER SESSION: CLINICAL - Adrenal Tumor Cases (1:30 PM-3:30 PM)

Title Major Diagnostic Pitfall and Challenging Management of a Rare Condition -- Pheochromocytoma

Author String J Bukowczan, J Murray, SHS Pearce, R Nayar
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Body

We report a rare case of pheochromocytoma mimicking acute abdomen. complicated by acute kidney and liver injuries requiring advanced treatment and extensive multidisciplinary team approach.

Background: 37 year old female presented acutely via Emergency Department with sudden onset severe abdominal pain radiating to her lower back, nausea, vomiting and headache. Positive signs and symptoms suggestive of peritonitis prompted CT abdomen. The patient had a sudden systolic BP rise to 220 mmHg and simultaneously oxygen saturations drop to 88% despite maximal therapy. Patient had a VF cardiac arrest but sinus rhythm was restored after few minutes of resuscitation. However, pulmonary edema with hypertensive crisis persisted. Intravenous furosemide and metoprolol given immediately in ITU lowered patient's blood pressure to 90/20 mmHg. Urgent CT abdomen revealed large right adrenal mass suggestive of pheochromocytoma. Plasma metadrenaline levels were > 25,000 pmol/L (n: 80 - 510 pmol/L). The patient was immediately commenced on phenoxybenzamine.

Delayed diagnosis due to unusual presentation resulted in multi organ failure. Acute kidney injury with creatinine level of 950 mmol/l (n: 58 - 96 mmol/L) was most likely due to acute tubular necrosis precipitated by exaggerated blood pressure changes due to unopposed beta receptor blockade as well as uncorrected hypovolaemia. It warranted urgent renal replacement therapy with haemodialysis daily until further development of polyuric phase and creatinine level of 127mmol/l off haemodialysis. The same mechanism contributed to acute ischaemic hepatitis reflected by transient dramatic rise of alanine transaminase to more than 5000 Units/l (n: 0 - 34 Units/L). Vigorous medical treatment in ITU resulted in reversal of acute hypoxic liver injury with normalization of liver function tests within 21 days.

After stabilization of patient's condition further tertiary centre referral was obtained. MIBG scan confirmed uptake in a right adrenal mass and additionally did not show any evidence of metastatic disease. Patient is currently awaiting surgery.

Conclusion: This case report highlights a major medical pitfall in diagnosis of pheochromocytoma. Acute onset abdominal symptoms posed a real diagnostic challenge We suggest maintaining high index of suspicion for pheochromocytoma in haemodynamically unstable patients presenting with acute abdomen.

Nothing to Disclose: JB, JM, SHSP, RN

Pub # P2-667

Session Information POSTER SESSION: CLINICAL - Adrenal Tumor Cases (1:30 PM-3:30 PM)

Title Metastatic Pheochromocytoma Diagnosed by Bone Marrow Biopsy after a Novel Presentation with Anemia and Thrombocytopenia

Author String M Grotzke
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Body Background: Pheochromocytomas are rare neuroendocrine tumors. The multiple effects of catecholamines result in a wide variety of presentations. We discuss a novel case of metastatic pheochromocytoma presenting with anemia and thrombocytopenia and diagnosed by bone marrow biopsy.
Clinical Case: A 21 year-old female presented to an outside hospital with pleuritic chest pain. Chest CT incidentally showed a right adrenal mass. Although normotensive, she was initiated on phenoxybenzamine and discharged home. She presented again several weeks later for syncope and hypotension resolving with cessation of the drug. She reported a 15 pound weight loss and 2 month history of weakness, abdominal pain, nausea, and vomiting. Anemia and thrombocytopenia were noted, prompting bone marrow biopsy. She was then referred to our institution.
Laboratory studies showed a hemoglobin of 9.1 g/dL (n12.1-15.9), platelets 57 k/uL (n177-406), alkaline phosphatase 380 U/L (n38-126), aspartate aminotransferase 67 U/L (n14-50), lactate dehydrogenase 1761 U/L (n300-600), metanephrines and normetanephrines (0.92 nmol/L, n0-0.49, and 6.64 nmol/L, n0-0.89). 24hr urine with dopamine 411 mcg/gCRT (n0-250), epinephrine <3 mcg/gCRT (n0-20), metanephrines 189 mcg/gCRT (n0-300), normetanephrines 4526 mcg/gCRT (n0-400), norepinephrine 17 mcg/gCRT (n0-45), homovanillic acid 108 mg/gCRT (n0-8), and vanillylmandelic acid 129 mg/gCRT (n0-6). CT imaging showed a 13.4cm right adrenal mass, abdominal lymphadenopathy, hepatomegaly with metastatic lesions and bifrontal subdural masses. Core bone marrow biopsy showed extensive replacement of marrow by atypical cells staining positive for synaptophysin and chromogranin, negative for cytokeratin, CD3, and CD20. Morphology was consistent with metastatic pheochromocytoma.
Cyclophosphamide, vinblastine, and dacarbazine (CVD) chemotherapy was started (1). Following the second cycle of CVD therapy, imaging revealed interval development of hepatic infarction, anasarca, and right pleural effusion. Laboratory data revealed elevation of serum metanephrines and normetanephrines above pre treatment values. The patient died 5 months after initial presentation.
Conclusion: Rare reports of anemia at the time of presentation exist, however pheochromocytoma presenting with thrombocytopenia has not previously been reported. This case of metastatic pheochromocytoma presenting with anemia and thrombocytopenia alerts the clinician to a novel finding in malignant pheochromocytoma.

(1) Adjalle R, Plouin PF, Pacak K, Lehnert H. Treatment of malignant pheochromocytoma. *Horm Metab Res* 2009 Sep; 41(9):687-96.

Nothing to Disclose: MG

Pub # P2-668

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Thyroid Cancer (1:30 PM-3:30 PM)

Title Thyroid Cancer Cell Lines Acquire Resistance to PLX4032 by an Increase in Receptor Tyrosine Kinase Activity

Author String C Montero-Conde, JM Dominguez, JA Knauf, A Viale, JA Fagin
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Body Patients with radioactive iodine-refractory (RAIR) thyroid cancer have a high mortality, particularly if positive on [18F] fluorodeoxyglucose PET scans. More than 60% of these tumors harbor the activating BRAF mutation V600E (1). PLX4032 is a novel class I RAF selective inhibitor that showed ~80% response rates in phase I clinical trial of BRAF (+) melanoma patients. However drug resistance developed 2-18 months from the initial response (2). We compared the pharmacokinetics of PLX4032 in thyroid cancer cell lines harboring BRAF^{V600E} (KTC1, BCPAP, SW1736, HTH104, 8505C, BHT101, T235) with melanoma cell lines with the same genotype (Malme3M, SK-Mel28, SK-Mel1, SK-Mel19). Five of seven thyroid cancer cell lines had IC₅₀ [ge] 2 [micro]M, whereas the melanoma lines had IC₅₀ of ~0.1 [micro]M. Interestingly, the thyroid lines had a pERK rebound beginning 6 h after PLX4032 treatment, whereas melanoma cells showed a sustained inhibition of MAPK signaling through 72 h (3). Re-addition of PLX4032 did not re-inhibit MAPK signaling in the thyroid cancer cell lines, consistent with rapid acquisition of resistance to the compound. Transcriptome profiling of SW1736 (thyroid) and SK-MEL28 (melanoma) cells at baseline, 1, 6 and 48 h after treatment with PLX4032 revealed several gene clusters with significantly different expression kinetics. PDGFR β was one of the transcripts with a significant expression increase after treatment in SW1736 compared to SK-MEL28 cells which was confirmed by Western blotting. Similar findings were observed after exposure of cells to the MEK inhibitor AZD6244. Moreover, phospho-RTK array analysis of SW1736 revealed increased phosphorylation of ErbB3 (HER3) in PLX4032-treated vs untreated cells, in addition to other less intense pRTK changes. In conclusion, after exposure to small molecule MAPK pathway inhibitors, BRAF (+) thyroid cancer cells are reprogrammed to overexpress a panel of RTKs, which individually or collectively are likely to mediate relative refractoriness to the action of these compounds. Whereas RTK overexpression has been described as a possible mechanism of secondary resistance to PLX4032 in melanomas, in thyroid cancer this feedback response takes place acutely, and is likely to mediate primary resistance to these compounds (4).

- (1) Ricarte-Filho JC et al., Cancer Res 2009; 69(11): 4885-93.
- (2) Flaherty KT et al., N Engl J Med 2010; 363(9): 809-19.
- (3) Joseph EW, et al. Proc Natl Acad Sci U S A 2010; 107(33): 14903-8.
- (4) Nazarian R, et al. Nature 2010; 468(7326): 973-7.

Sources of Research Support: NIH grants CA50706 and CA72598 (JAF) and the Spanish Ministry of Science and Innovation (MICCIN-Programa nacional de movilidad de recursos humanos del plan nacional de I-D+I 2008-2011) grant BMED 2008-0659 (CM-C). We thank Gideon Bollag at Plexxikon Inc. for providing PLX4032.

Nothing to Disclose: CM-C, JMD, JAK, AV, JAF

Pub # P2-669

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Thyroid Cancer (1:30 PM-3:30 PM)

Title Targeting SRC and Focal Adhesion Kinase (FAK) in Thyroid Cancer

Author String CM Chan, G Lund, V Sharma, B Haugen, R Schweppe
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Body Approximately 1, 700 patients die each year from advanced thyroid cancer. These patients may not respond to traditional treatments necessitating the identification of other potential therapeutic strategies. The Src-Focal Adhesion Kinase (FAK) signaling module plays an important role in promoting cancer cell growth and invasion. We recently demonstrated the activation of Src and FAK in thyroid cancer cell lines and have shown that FAK is activated and overexpressed in thyroid tumors (1). To further study the role of the Src-FAK pathway, Src activity was inhibited using dasatinib (BMS-354825), a selective dual Src/Abl kinase inhibitor that is currently being tested in solid tumors and is FDA-approved for the treatment of chronic myelogenous leukemia. The effects of Src inhibition on thyroid cancer growth was studied in a panel of authenticated papillary and anaplastic thyroid cancer (PTC and ATC) cell lines (BCPAP, SW1736, K1, and 8505C). Cells were treated with increasing doses of dasatinib (18.75 nM to 1.25 [μ M]) for 3 days and proliferation was measured using the sulforhodamine B assay. BCPAP (PTC) and SW1736 (ATC) cells were most sensitive to dasatinib (IC₅₀s < 0.08 [μ M]); K1 (PTC) cells were intermediate (IC₅₀ = 0.4 [μ M]), and 8505C (ATC) cells were most resistant (IC₅₀ = 2.7 [μ M]). The effects of Src inhibition on invasion was tested in the BCPAP and 8505C cells, which exhibited high or low sensitivity to Src inhibition, respectively. Invasion of the BCPAP cells was blocked by nanomolar concentrations of dasatinib (90% at 50 nM), while the 8505C cells were also sensitive (60% at 50 nM). Consistent with the role of Src and FAK in growth and invasion, we found a corresponding inhibition of Src activity (pY416) and FAK phosphorylation (pY861) at low nanomolar concentrations (50 nM) in all cell lines tested. Phosphorylation of the downstream adaptor molecule p130Cas (pY410) was also inhibited at similar concentrations and kinetics. FAK has important cellular functions as a scaffolding protein as well as a signaling kinase. To test the scaffolding functions of FAK, we inhibited FAK protein expression using short inhibitory RNAs (shRNAs) in the Src-sensitive BCPAP cells and found that knockdown of FAK expression blocked proliferation by 50-65% at 6 days. In summary, our results indicate an important role for the Src-FAK signaling pathway in thyroid cancer growth and invasion, potentially via key functions of FAK.

(1) Schweppe RE, et al., JCEM 2009; 94(6):2199-203

Disclosures: BH: Speaker, Genzyme Corporation; Study Investigator, Veracyte, Inc. Nothing to Disclose: CMC, GL, VS, RS

Pub # P2-670

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Thyroid Cancer (1:30 PM-3:30 PM)

Title Development of a Novel Model of Distant Metastases in Thyroid Cancer and Assessment of NF[κ]B Signaling on Establishment and Progression of Metastases

Author String G Lund, K Bauerle, L Pike, C Chan, B Haugen, R Schweppe
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Body The development of distant thyroid cancer metastases, especially to the bone, is often correlated with a poor prognosis. A robust in vivo model of thyroid cancer metastasis, especially bone, has been lacking. We have therefore developed a novel experimental metastasis model for papillary and anaplastic thyroid cancer. Three authenticated thyroid cancer cell lines were used in an intracardiac injection model in athymic nude mice: 8505C (anaplastic), BCPAP (papillary) and K1 (papillary). All cell lines have been engineered to express GFP-luciferase in order to monitor metastases in real time using bioluminescence (IVIS) imaging. Thyroid cancer cells are injected into the left ventricle of the heart to model widespread dissemination of cancer cells. Mice are imaged immediately after intracardiac injection to ensure successful injection into the left ventricle and the systemic circulation of cancer cells. Using this approach, we have shown that injection of 1×10^5 8505C or BCPAP cells results in the development of clinically relevant metastases to the bone, brain and soft tissue in all mice by 1-3 weeks (16/16 take rate for the 8505C mice and 18/18 take rate for the BCPAP mice). Notably, the K1 cells did not form any tumors using this model. The NF κ B pathway is important for thyroid cancer development, growth, invasion and metastases. We therefore used our intracardiac injection model to study the effect of NF κ B signaling on thyroid cancer metastases. We used 8505C and BCPAP cells in which the NF κ B pathway had been genetically inhibited by retroviral transduction of a dominant negative I κ B α (mI κ B α) or a vector control. Interestingly, inhibition of total metastatic burden by mI κ B was not achieved in the 8505C mice until 4 weeks ($p=0.005$), whereas the BCPAP mI κ B mice exhibited significantly lower overall metastatic burden as early as 1 week ($p=0.0002$), suggesting distinct signaling mechanisms. At 4 weeks, NF κ B inhibition significantly reduced overall tumor burden by 72.4% in mice injected with 8505C cells ($p=0.005$) and 84.3% in mice injected with BCPAP cells ($p=0.0014$). NF κ B inhibition improved 5 week survival of mice for both cell lines (8505C: 50% survival for vector vs. 92% for mI κ B; BCPAP: 82% survival for vector vs. 100% for mI κ B). In summary, we have developed a novel model of cancer metastases for both anaplastic and papillary thyroid cancer and have demonstrated the importance of NF κ B in the establishment and progression of thyroid cancer metastases.

Disclosures: BH: Speaker, Genzyme Corporation; Study Investigator, Veracyte, Inc. Nothing to Disclose: GL KB, LP, CC, RS

Pub # P2-671

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Thyroid Cancer (1:30 PM-3:30 PM)

Title Metformin Activates AMPK and Inhibits Thyroid Cancer Cell Growth in an Oncogene-Specific Manner

Author String J Klubo-Gwiedzinska, J Costello, Jr, K Jensen, KD Burman, L Wartofsky, VV Vasko
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Body

Introduction: AMP-activated protein kinase (AMPK) is a central metabolic switch that governs glucose and lipid metabolism in response to alterations in intracellular energy levels. AMPK activation causes inhibition of cell growth by switching off the target-of-rapamycin (TOR) pathway. The PI3K/AKT/mTOR signaling cascade is activated in thyroid cancer and inhibition of this signaling emerged as a possible therapeutic strategy for thyroid cancer patients. The anti-diabetic drug Metformin reduces mTOR activity and inhibits the growth of a wide variety of tumors in an AMPK dependent manner.

Objective: We evaluated AMPK expression in thyroid cancer and examined the effects of Metformin on thyroid cancer cell growth.

Methods: Papillary thyroid cancer derived cells lines (TPC1 and BCPAP) were used in this study. Treatment with increasing concentration of Metformin (50[μ]M - 5mM) was performed for 72 hours. Activation of AMPK and expression of apoptotic markers were analyzed by immunoblotting. Cell viability and cell proliferation assays were performed. Human tissue samples from benign and malignant thyroid lesions were examined by immunostaining.

Results: AMPK expression, but not AMPK activation, was detected in examined thyroid cancer cell lines. Treatment with Metformin for 72 hours resulted in AMPK activation in a dose dependent manner. Metformin treatment was associated with inhibition of thyroid cancer cell growth. In RET/PTC positive TPC1 cells treatment with Metformin had no effect on pAkt. In BRAF positive BCPAP cells treatment with Metformin was associated with loss of pAkt. In human thyroid samples pAMPK was not detected in normal thyroid or in hyperplastic thyroid nodules. Focal staining with pAMPK was noted in Hashimoto's thyroiditis. Immunostaining with anti-pAMPK antibody was negative in most thyroid cancers harboring RET/PTC or BRAF mutations. However, positive pAMPK staining was found in papillary cancers with lymphocytic infiltration of tumor stroma and in Hurthle cell carcinoma.

Conclusion: AMPK signaling is not activated in PTC derived thyroid cancer cell lines. Metformin activates AMPK and inhibits thyroid cancer cells growth in an oncogene specific manner. AMPK signaling is inhibited in most human thyroid tumors, but oncocytic thyroid lesions demonstrate intrinsically high level of pAMPK. Defining molecular and morphological characteristics which can predict thyroid cells response to AMPK activating drugs is an important goal for future studies.

Nothing to Disclose: JK-G, JC, KJ, KDB, LW, VVV

Pub # P2-672

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Thyroid Cancer (1:30 PM-3:30 PM)

Title Metastatic Characteristics and Desensitization to Radiotherapy Depends on PI3K and HIF-1 Signaling in Thyroid Carcinomas

Author String N Burrows, M Babur, J Resch, S Ridsdale, J Williams, KJ Williams, G Brabant
University of Manchester, Manchester, UK; The Christie, Manchester, UK

Body The PI3K and HIF-1 signaling pathways are associated with aggressive disease, desensitisation to chemo/radiotherapy and poor prognosis in many cancers. HIF-1 is a transcription factor that is activated under low oxygen (hypoxia) or by oncogenic signaling pathways such as PI3K/AKT. Little is known about the role of HIF-1 in thyroid carcinoma. Clinically HIF-1 has been linked with aggressive phenotype and activity and expression related to aggressiveness in a range of thyroid carcinoma cell lines (1). We aimed to determine the importance of the PI3K and HIF-1 pathways in a) metastatic characteristics and b) desensitisation to radiotherapy.

To assess metastatic characteristics, migration and cell-spreading assays were performed under various oxygen-tensions. Inhibition of PI3K and HIF-1 was achieved using PI3K-inhibitors PI-103 and GDC-0941, by over-expressing PTEN in PTEN-null cells and by use of a dominant-negative variant of HIF-1 α (dnHIF). Mice bearing anaplastic and eGFP-expressing follicular thyroid carcinomas were treated with PI-103 and GDC-0941. Spontaneous lung metastasis was confirmed by viewing eGFP+ colonies cultured from digested lung tissue and immuno-blotting for eGFP. For irradiation studies, cells were exposed to 2, 4, 6GY radiation. PI3K and hypoxia increased cell migration and spreading. Inhibition of PI3K and HIF-1 reduced cell migration, spreading, PI3K and HIF-1 activity and expression of HIF-1 target genes (CA9, VEGF, LDHA, GLUT1). Furthermore, differential expression of AKT isoforms were observed in hypoxia which may modulate cell migration. PI-103 and GDC-0941 reduced PI3K and HIF-1 activity and downstream targets in follicular and anaplastic tumors. Mice bearing dnHIF-follicular tumors and those treated with GDC-0941 had reduced lung metastasis. Radiation induced HIF-1 activity and expression. GDC-0941 combined with radiation blocked HIF-1 induction, prolonged DNA double-strand breaks and reduced clonogenic survival suggesting a radio-sensitising effect.

These data highlight the importance of the PI3K and HIF-1 pathways in metastasis and treatment response in thyroid carcinoma. The results are first to show PI3K-inhibitors may be important therapeutically in a) reducing metastasis and b) increasing therapeutic response to radiotherapy. With the known desensitizing effects of PI3K and HIF-1 activity on radiotherapy, combining radiation with a PI3K-inhibitor may improve both the therapeutic response as well as reducing metastatic burden.

(1) Burrows N et al., *Endocr Relat Cancer*; 2010; 17(1):61-72

Nothing to Disclose: NB, MB, JR, SR, JW, KJW, GB

Pub # P2-673
Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Thyroid Cancer (1:30 PM-3:30 PM)
Title Distinctive Features of Autophagy in Thyroid Hürthle Cell Tumors
Author String JU Lee, MK Kim, JH Yim, K-H Jung, MH Lee, SE Lee, KS Kim, TY Kim, YK Shong, S-H Lee, JM Kim, M Shong, YS Jo
Daejeon St Mary's Hospital, The Catholic University of Korea, Daejeon, Korea; Chungnam National University School of Medicine, Daejeon, Korea; University of Ulsan College of Medicine, Asan Medical Center, Seoul, Korea; Chungnam National University School of Medicine, Daejeon, Korea
Body Abstract Embargoed.

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- (2) Asa SL et al., *J Clin Pathol* 2004; 57:225
- (3) Cibas ES et al., *Thyroid* 2009; 19:1159
- (4) Maximo V et al., *J Pathol* 2000; 192:561
- (5) Gasparre G et al., *Proc Natl Acad Sci U S A* 2007; 104:9001
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Sources of Research Support: Grant of the Korea Healthcare Technology R&D Project, Ministry for Health, Welfare and Family Affairs, the Republic of Korea (A080744 & A100588).

Nothing to Disclose: JUL, MKK, JHY, K-HJ, MHL, SEL, KSK, TYK, YKS, S-HL, JMK, MS, YSJ

Pub # P2-674

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Thyroid Cancer (1:30 PM-3:30 PM)

Title Different CXCR4 Expression According to Histologic Subtype of Papillary Thyroid Carcinoma

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Body Functional chemokine (C-X-C motif) receptor 4 (CXCR4) stimulates proliferation and chemotaxis of various tumor cells in response to endogenous stromal cell-derived factor-1 (SDF-1)/CXCL12 chemokine. CXCR4 is well known to be over-expressed in papillary thyroid carcinoma (PTC) and more extensively in anaplastic thyroid carcinoma (ATC). The aim of this study was to evaluate whether the expression of CXCR4 is different by histological subtype of PTC and to elucidate the relationship between the expression of CXCR4 and known clinicopathologic factors. We prepared 127 PTC tissue samples from 130 patients of PTC using tissue microarray technique, and the level of CXCR4 expression in tumors and surrounding normal region was assessed using immunohistochemical staining for CXCR4 molecule. The expression of CXCR4 showed different patterns according to histological subtype of PTC ($p < 0.001$). A strong expression of CXCR4 was observed more frequently in the region of poorly differentiated region of PTC (81.0%) than in classical papillary microcarcinoma (50.0%). Strong CXCR4 expression was less frequent in follicular variant (33.9%) and diffuse sclerosing variant (14.3%) of PTC. Other pathological features of tumor such as tumor size, the existence of extrathyroidal invasion, lymph node metastasis, multiplicity and lymphocytic infiltration were not correlated with the incidence of a strong CXCR4 staining pattern. CXCR4 expression showed a distinct pattern according to histological subtype of PTC although not associated with other clinicopathological parameters. CXCR4 expression seems to be a possible marker representing different prognosis of each PTC variant regardless of initial cancer stage.

Sources of Research Support: Yonsei University College of Medicine faculty research grant 2010 (No. 6-2010-0137).

Nothing to Disclose: DyS, MKL, KHN, WYC, EJJ

Pub # P2-675

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Thyroid Cancer (1:30 PM-3:30 PM)

Title N-t-Boc-Hexylenediamine Derivative of 7-(O)-Carboxy-Methyl Daidzein Inhibits the *In Vitro* Growth of Human Thyroid Cancer through Estrogen Receptor B-Dependent Pathways Involving the Formation of Reactive Oxygen Species

Author String D Somjen, M Grafi-Cohen, G Weisinger, O Sharon, Z Kraiem, E Izkhakov, F Kohen, N Stern
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Body Thyroid cancer incidence is up to three fold higher in women than in men, suggesting the possible involvement of estrogens in its pathogenesis. The present study investigated the effect of a novel isoflavone-derived anti-estrogenic compound developed in our laboratory, the N-t-boc-hexylenediamine derivative of 7-(O)-carboxymethyl daidzein [cD-tboc] in human thyroid cancer cells. First: the mRNA expression of estrogen receptor a and b (ERa and ERb) was confirmed in several human thyroid cancer cell lines, in human non-malignant, in goiterous cells and in papillary thyroid cancer cells harvested during thyroidectomy. All cell types expressed both ERa and ERb with a variably higher abundance of ERb over ERa. Second: DNA synthesis and creatine kinase (a marker of estrogenic genomic response) were increased in response to estradiol-17b (E2), the ERa agonist PPT as well as the ERb agonist DPN. Third: as determined by DNA synthesis, the XTT assay and direct microscopic visualization, cD-tboc markedly inhibited cell growth in all types of human thyroid cancer by-60-90%, be it of cell line- or patient-derived origin and also slowed down, albeit to a lesser extent, the growth of non-cancerous human thyroid cells (0-50%). Very significantly, cD-tboc abolished E2-induced cell growth in cancer cells, but only partially in goiter and normal cells (70 vs. 45%). Fourth: functionally critical for the growth-inhibitory effect of cD-tboc was its ability to increase ROS formation, since inhibition of NADPH-oxidase activity by DPI not only abolished ROS formation, but also partially inhibited the cytotoxic effects of cD-tboc. Fifth: cD-tboc could not induce cancer cell death when ERb was inactivated either by co-incubation with its antagonist PTHPP (10 vs 70%) or human anaplastic thyroid cancer cell line transfected with ERb SiRNA (5 vs 70%) but not ERa SiRNA. In the latter cells, the expression of ERb was markedly suppressed (0 vs 70%). This is the first evidence that cD-tboc acts as an anti human thyroid cancer agent *in vitro* in a variety of cell types (including cancer cells removed from human thyroid cancer patients) via ERb- dependent mechanism(s) involving ROS formation.

Nothing to Disclose: DS, MG-C, GW, OS, ZK, EI, FK, NS

Pub # P2-676

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Thyroid Cancer (1:30 PM-3:30 PM)

Title The Effects of Reversine, a 2,6-Disubstituted Purine, on Poorly Differentiated and Follicular Thyroid Cancer Cell Lines

Author String S-C Hua, T-C Chang, Y-R Lee
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Body Because of the current treatment dilemma in incurable differentiated thyroid cancer (DTC) and poorly differentiated thyroid cancer (PDTC), a novel and effective treatment is urgently needed. Reversine, a small synthetic purine analogue (2,6-disubstituted purine), has been shown effective in tumor suppression (1,2). In this study, we *in vitro* evaluated the anti-tumor effects of reversine on proliferation, cell cycle, and apoptosis in human PDTC (SW579) and follicular thyroid cancer (WRO) cell lines, respectively. Treatment of these two cell lines with reversine inhibited proliferation in a time- and dose-dependent manner by MTT assay. G2/M accumulation was demonstrated by cell cycle analysis in both cell lines. Only in SW579, reversine induced apoptosis with caspase-3 and caspase-8 activation, but not caspase-9. Application of pan-caspase inhibitor before treatment of reversine attenuated cell death. In conclusion, our data demonstrated that reversine is effective in growth inhibition of thyroid cancer cell lines. Tumor suppression is via cell cycle arrest both in DTC and PDTC cell lines, but apoptosis is induced only in PDTC cell lines by mitochondria-independent pathway. Reversine is worthy further investigation in clinical therapeutics research.

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Sources of Research Support: Chia-Yi Christian Hospital Grant (R98-9).

Nothing to Disclose: S-CH, T-CC, Y-RL

Pub # P2-677

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Thyroid Cancer (1:30 PM-3:30 PM)

Title Expression and Biological Role of EpCAM in Anaplastic Thyroid Cancer

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Body Anaplastic thyroid cancer (ATC) is among the most lethal human malignancies with a mean survival rate of approximately 4-12 months. Treatment options with chemotherapy are limited due to fast rate of tumor growth and high metastatic potential. Epithelial cell adhesion molecule (EpCAM) is a 40 KD glycoprotein which functions as epithelial cell adhesion molecule and mediates Ca²⁺-independent homotypic cell adhesion. EpCAM over expression has been previously demonstrated in differentiated thyroid cancers but the evidence supporting its presence in anaplastic thyroid cancer is lacking. In this study we report that EpCAM is highly expressed in anaplastic thyroid cancer cell lines ACT1, T235 and T238 ranging from ~3 to 90%. We also report that this marker is not expressed in adult normal thyroid TAD-2 cell line. We used flow cytometric analysis to study the expression of EpCAM. The ATC-derived cell lines used in this experiment were free of any cross contamination with other cells as they were previously analyzed by DNA profiling. Epithelial cell adhesion molecule (EpCAM) is expressed by a broad variety of carcinoma cells. To our knowledge this is the first report demonstrating the presence of EpCAM in anaplastic thyroid cell lines. We are also studying the further role of EpCAM as a cancer stem cell marker, its role in in-vivo tumorigenesis and invasiveness of ATC cells and its potential role as a target for drug/gene therapy strategies.

Nothing to Disclose: DB, EB-D, LG, KL, FF

Pub # P2-678

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Thyroid Cancer (1:30 PM-3:30 PM)

Title Liver Detargeting of Adenoviral Vectors by Polymer Coating after Systemic Delivery Using the Sodium Iodide Symporter (NIS) as Reporter Gene

Author String GK Grunwald, A Vetter, K Klutz, N Wunderlich, R Senekowitsch-Schmidtke, PS Holm, E Wagner, B Goke, M Ogris, C Spitzweg
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Body We have recently demonstrated induction of tumor-selective accumulation and therapeutic efficacy of radioiodine in different tumor models after systemic non-viral sodium iodide symporter (NIS) gene delivery demonstrating the high potential of NIS in its dual function as reporter and therapy gene. Recombinant adenovirus-mediated gene therapy is a powerful strategy in cancer treatment and represents another promising method for NIS gene delivery. However, high promiscuity due to widespread expression of the coxsackie- and adenovirus receptor (CAR) and significant pooling in the liver are major limitations for its clinical application after systemic delivery. Polymer coating of adenoviral vectors represents a promising tool to detarget adenoviral vectors away from CAR and liver towards tumor-specific targets.
We therefore generated novel polyviroplexes by non-covalent modification of a replication-deficient adenovirus carrying the *h*NIS gene linked to the CMV-promoter (Ad5-CMV/NIS) with synthetic and low toxic fifth-generation poly(amidoamine) dendrimers (PAMAM-G5). In this study we characterized PAMAM-G5 coated Ad5-CMV/NIS and analyzed the transduction efficacy and altered tropism of these polyviroplexes compared to uncoated Ad5-CMV/NIS using NIS as reporter gene.
In vitro experiments with PAMAM-G5 coated adenovirus demonstrated enhanced transduction efficacy in a CAR-negative cell line (U87MG), which showed a 5.5-fold increase in perchlorate-sensitive iodide uptake using polyviroplexes as compared to uncoated Ad5-CMV/NIS.
In vivo experiments showed high levels of functional NIS protein expression in the liver of nude mice 4 d after i.v. injection of 1×10^9 PFU Ad5-CMV/NIS as shown by ^{123}I gamma camera imaging due to scavenging of the adenovirus by Kupffer cells and uptake by hepatocytes. In comparison to uncoated Ad5-CMV/NIS, systemic injection of polyviroplexes resulted in a significant reduction of hepatic iodide accumulation by 70%. In addition, qPCR and immunohistochemical analysis of liver tissue revealed high NIS expression levels in mice infected systemically with uncoated Ad5-CMV/NIS whereas mice infected with PAMAM-G5 coated Ad5-CMV/NIS showed very low levels of NIS expression in the liver.
In conclusion adenovirus coating with synthetic polymers represents a very efficient way to overcome adenoviral pooling in the liver, one of the most limiting barriers for systemic adenovirus-mediated gene delivery.

Nothing to Disclose: GKG, AV, KK, NW, RS-S, PSH, EW, BG, MO, CS

Pub # P2-679

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Thyroid Cancer (1:30 PM-3:30 PM)

Title Therapeutic Potential of Stem Cell-Mediated Sodium Iodide Symporter (NIS) Gene Delivery in Liver Cancer

Author String K Knoop, M Kolokythas, K Klutz, MJ Willhauck, N Wunderlich, D Draganovici, C Zach, FJ Gildehaus, G Boning, B Goke, E Wagner, PJ Nelson, C Spitzweg
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Body Due to its dual role as reporter and therapy gene, the sodium iodide symporter (NIS) allows non-invasive imaging of functional NIS expression by ^{123}I -scintigraphy or ^{124}I -PET imaging before the application of a therapeutic dose of ^{131}I . NIS expression provides a novel mechanism for the evaluation of mesenchymal stem cells (MSCs) as gene delivery vehicles for tumor therapy.

In the current study we stably transfected human bone marrow derived CD34⁺ MSCs with a NIS-expressing plasmid (CMV-NIS-pcDNA3) (NIS-MSC) followed by analysis of MSC-mediated NIS expression and cytotoxicity of ^{131}I .

NIS-MSCs revealed a 12-fold increase in perchlorate-sensitive iodide uptake activity due to high levels of functional NIS protein expression as compared to wild-type (WT)-MSCs. After establishment of subcutaneous Huh7 xenografts in nude mice, NIS-MSCs were injected via the tail vein 3 times in 4-day intervals, followed by analysis of radioiodine distribution using ^{123}I -gamma camera and ^{124}I -PET imaging as well as *ex vivo* gamma counting. After systemic NIS-MSC injection Huh7 xenografts accumulated approximately 9.5 % ID/g ^{123}I with a biological half-life of 4 h, resulting in a tumor absorbed dose of 43.7 mGy/MBq. In *ex vivo* biodistribution experiments only low or now significant iodide uptake was detected in non-target organs like liver, lung, kidney and spleen. To examine the therapeutic effect of NIS-mediated radioiodine therapy, 55.5 MBq ^{131}I was administered 48 h after the last of three MSC-applications in 2-day intervals. After two of these treatment cycles followed by a further single MSC injection with a final ^{131}I application 48 h later, Huh7 xenografts showed a pronounced delay in tumor growth which was associated with improved survival. In contrast, mice treated with NIS-MSCs followed by saline-application or mice treated with WT-MSCs followed by ^{131}I -application or saline treated mice showed an exponential tumor growth. In addition, immunofluorescence analysis showed markedly reduced proliferation and decreased blood vessel density of NIS-MSC/ ^{131}I treated tumors in contrast to control tumors.

Taken together, our results convincingly show tumor-specific accumulation and therapeutic efficacy of radioiodine after MSC-mediated NIS gene delivery in HCC tumors, opening the exciting prospect of NIS-mediated radionuclide therapy of metastatic cancer using MSCs as gene delivery vehicles.

Nothing to Disclose: KK, MK, KK, MJW, NW, DD, CZ, FJG, GB, BG, EW, PJN, CS

Pub # P2-680

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Thyroid Cancer (1:30 PM-3:30 PM)

Title Thr300Ala *ATG16L1* Polymorphism Influences Susceptibility to, and the Prognosis of, Epithelial Cell[en dash]Derived Thyroid Carcinoma

Author String A Huijbers, TS Plantinga, M Oosting, LAB Joosten, KKH Aben, BA Kimeney, MG Netea, ARMM Hermus, RT Netea-Maier
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Body **BACKGROUND** Autophagy influences both carcinogenesis and modulation of inflammation. Recent data suggest that loss of autophagy protein ATG16L1 enhances endotoxin-induced interleukin-1 β (IL-1 β) production. IL-1 β has been previously found to have antineoplastic effects in thyroid malignancies. We hypothesized that a missense polymorphism in *ATG16L1* autophagy gene (Thr300Ala) results in changes in function of the molecule, and influences susceptibility to and prognosis of epithelial cell derived thyroid carcinoma (TC).
OBJECTIVES i. to assess functional differences between the 300Thr and 300Ala allele of *ATG16L1*; and ii. to assess whether *ATG16L1*/Thr300Ala polymorphism influences susceptibility to and prognosis of TC.
PATIENTS AND METHODS The *ATG16L1* genotype was analyzed by PCR in 139 patients with TC and of 136 healthy controls. In the control group peripheral blood mononuclear cells were stimulated with the NOD2 ligand muramyl dipeptide (MDP). The function of ATG16L1 was assessed by measuring IL-1 β 24h later. Within the patients group we analyzed the genotype-phenotype associations.
RESULTS MDP-stimulation of IL-1 β production was significantly higher (by 75% and 120% in heterozygous and homozygous individuals respectively, $p < 0.05$) in individuals bearing the GG/GA (300Ala) allele compared to those bearing AA (300Thr) allele. These data support a gene-dosage or a dominant effect of the 300Ala variant on the function of the molecule. The difference in genotype frequencies between the patients and control groups were analyzed in a gene dosage dependent model using logistic regression and in a dominant model using [chi]² analysis. A significant gene-dosage effect was seen for increasing numbers of the 300Ala (G) allele (OR=0.46, $p=0.041$). Analysis in a dominant model also showed a protective effect of AG/GG genotype compared to the AA genotype (OR=0.51, $p=0.025$). Patients carrying the AG/GG genotype had a higher chance of successful I131 ablation ($p=0.017$) and required a lower cumulative I131 dose to achieve remission ($p=0.014$) than the patients carrying the AA genotype.
CONCLUSIONS The Thr300Ala *ATG16L1* polymorphism has important consequences for the function of the molecule, with significantly higher IL-1 β release in individuals bearing the 300Ala variant. We also demonstrate that the 300Ala (G) allele was associated with both a lower susceptibility to TC and lower I131 dose required to achieve remission in a cohort of Dutch patients.

Nothing to Disclose: AH, TSP, MO, LABJ, KKHA, BAK, MGN, ARMMH, RTN-M

Pub # P2-681

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Thyroid Cancer (1:30 PM-3:30 PM)

Title HSV2 Infection Targets Thyroid Gland and Affects Expression of DNA Repair Molecules in Thyroid Cells

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Body

Introduction: Thyroid cancer cells express viral entry receptors and are permissive to the human herpes viruses (HSV). In epithelial cells, HSV can modulate the cellular response to DNA damage through inhibition of the cellular histone ubiquitin ligase RNF8. We hypothesized that HSV can also target thyroid cells and affect expression of genes controlling DNA damage response.

Methods: Cotton rats (*Sigmodon hispidus*) were inoculated intra-vaginally with HSV-2. Thyroid gland was harvested on days 1, 4, 21 post-infection (p.i.) and on day 28 (after reactivation by dexamethasone). Expression of HSV DNA polymerase, ICP34.5 and thymidine kinase (HSV-TK) was assessed by PCR. HSV capsid protein was assessed by immunostaining. Host cell response was examined by immunostaining with antibodies against IFN- β , NF[kappa]B, p-PKR and p-eIF2 α . DNA repair signaling was assessed with antibodies against ATM, pATM, H2X, γ H2X and RNF8.

Results: HSV-2 fully recapitulated human disease in cotton rats. Epithelial lesions developed at day 4 p.i. and spontaneously resolved by day 21. At the onset of clinically evident viral replication at the site of inoculation, HSV DNA polymerase, ICP34.5 and HSV-TK were detected in thyroid gland. Staining with HSV capsid protein was positive in thyroid cells. HSV-2 infection was not associated with changes in thyroid gland morphology. However, high expression of IFN- β and NF[kappa]B was detected in thyroid cells of infected animals. The activation of anti-viral signaling in thyroid cells was demonstrated by positive staining with p-PKR and p-eIF2 α antibodies. ATM kinase was activated, but RNF8 expression was decreased in infected thyroid cells. At day 21 p.i, there was no HSV-2 mRNA or protein in thyroid gland. Expression of IFN- β and NF[kappa]B was maintained, but staining with p-PKR and p-eIF2 α antibodies was negative. The level of RNF8 in thyroid cells remained low at day 21. HSV-2 reactivation on day 28 was associated with expression of viral protein in thyroid cells. During HSV-2 reactivation, the host cell response and DNA repair signaling were activated as seen at day 4.

Conclusion: Thyroid cells can be targeted by HSV-2. Activation of anti-viral signaling in thyroid cells was associated with subsequent cell clearance of infection. HSV-2 inhibits RNF8 and compromises the integrity of DNA repair signaling. These data suggest that HSV-2 infection may predispose thyroid cell to the mutagenic effects of other factors.

Nothing to Disclose: KJ, AP, AB, VV

Pub # P2-682

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Thyroid Cancer (1:30 PM-3:30 PM)

Title Regulatory T Cells and PD-1+ T Cells Are Enriched in Primary Tumors and Metastatic Lymph Nodes and Are Associated with More Severe Disease in Patients with Papillary Thyroid Cancer

Author String JD French, GR Kotnis, S Said, CD Raeburn, RC McIntyre, BR Haugen
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Body

Background: Patients with advanced papillary thyroid cancer (PTC) could benefit from innovative adjuvant therapies (e.g. immune-based therapies). Our previous studies revealed that the immune response to PTC is unproductive and may promote disease progression. Using archived surgical tissues, we showed that increasing frequency of regulatory T cells (Tregs) in PTC nodules was associated with higher incidence of lymph node metastases. The presence of immune-suppressive Tregs may partially explain the ability of PTC to evade immune destruction. Recent studies in melanoma revealed that T cells expressing Programmed Death-1 (PD-1) are enriched in tumors and show signs of exhaustion (CD27^{hi}, TNF α ^{lo}, IFN γ ^{lo}). We hypothesize that T cell exhaustion is utilized in patients with advanced PTC as a mechanism of immune evasion.

Methods: 38 patients with PTC undergoing thyroidectomy with or without neck lymph node dissection and 6 patients having secondary surgery for recurrent lymph node disease were included in this study. We sampled thyroid nodules, uninvolved (UILN), and tumor-involved lymph nodes (TILN) by *ex vivo* fine-needle biopsy. Following *in vitro* stimulation, lymphocytes were analyzed by flow cytometry. Patient-matched peripheral blood lymphocytes (PBL) served as a control.

Results: Tregs were enriched in primary tumors and TILN compared to PBL and UILN, respectively. Treg frequency was further enriched in TILN from patients with recurrent disease. PD-1+ T cells were increased in both primary tumors and in TILN, and level of PD-1+ T cells correlated directly with Treg frequency. Increasing PD-1+CD8+ T cell frequency in PTC nodules correlated with higher incidence of LN metastases ($r=0.8289$; $p=0.0341$). PD-1+CD4+ ($p=0.0439$) and PD-1+CD8+ ($p=0.0045$) T cell levels were significantly higher in TILN with evidence of extranodal invasion compared to non-invasive metastases. Phenotypic characterization revealed that PD-1+ T cells were primarily CD45RA- memory cells. Although these cells expressed high levels of IFN γ and TNF α , they failed to down-regulate CD27.

Conclusions: These studies identify Tregs and PD-1+ T cells as markers of immune suppression and more severe disease in patients with PTC. Although PD-1+ T cells maintain the ability to produce effector cytokines, their failure to down-regulate CD27 suggests that they may be partially exhausted. Future studies may identify PD-1-targeted immune-based therapies as a viable option for patients with advanced PTC.

Sources of Research Support: American Thyroid Association THANC 2009; American Cancer Society IRG #57-001-50; NIH/NCRR Colorado CTSI Grant #UL1 RR0 25780.

Disclosures: BRH: Speaker, Genzyme Corporation; Study Investigator, Veracyte, Inc. Nothing to Disclose: JDF, GRK, SS, CDR, RCM

Pub # P2-683

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Thyroid Cancer (1:30 PM-3:30 PM)

Title Chronic Lymphocytic Thyroiditis May Modulate Tumor Microenvironment and Elicit Antitumor Immune Response Influencing Clinical and Pathological Features of Differentiated Thyroid Carcinomas

Author String LL Cunha, EC Morari, S Nonogaki, FA Soares, J Vassallo, LS Ward
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Body Chronic lymphocytic thyroiditis (CLT) is frequently associated to differentiated thyroid cancer (DTC) but, although we and others have reported data suggesting that autoimmune activity against the gland may exert a protective effect on patients' outcome, the subject is still a matter of debate. Immune cells are important components of tumor microenvironment and CLT could elicit an intratumor immune response characterized by mixed composition that could be responsible for antitumor defense. In order to further investigate the influence of the immune response on the prognostic of DTC patients, we studied 266 DTC patients managed according to a standard protocol and followed-up for 43.50±33.29 months (median 12 a 298 months). According to their serum Tg levels and other evidences of recurrence/metastasis, patients were classified as no evidence-of-disease (NED=216 cases) or persistence of disease/recurrence (REC=50 cases including 10 deaths). We analyzed the presence of B-lymphocytes (CD20+), pan T-lymphocytes (CD3+), T-helper lymphocytes (CD4+), Cytotoxic T-lymphocyte (CD8+), regulatory T-lymphocytes (FoxP3+), and macrophage (CD68+) using immunohistochemistry. CLT was found in 31.57% of the DTC cases and was more frequent in smaller tumors (p=0.0110), cases with no extrathyroidal invasion (p=0.0028), and no metastasis at diagnosis (p=0.0026). In addition, a log-rank test confirmed that the absence of CLT was more frequent among patients who presented recurrences (p=0.00149). CLT occurred more frequently in cases which tumors presented B (p<0.0001) and T lymphocyte (p<0.0001), T helper lymphocyte (p<0.0001), cytotoxic T-lymphocyte (p<0.0001), regulatory T-lymphocytes (p<0.0001), and macrophage infiltration (p=0.0260). Macrophages (p=0.04963) and CD8+ cells were more frequent in NED patients (p=0.03119). Our result suggests that the presence of CLT is associated to a profile of defense immune cells recruitment. We conclude that, although an appropriated management of DTC patient is the most important and modifiable prognostic factor, the immune response profile may help explain the outcome of some DTC patients.

Nothing to Disclose: LLC, ECM, SN, FAS, JV, LSW

Pub # P2-684

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Thyroid Cancer (1:30 PM-3:30 PM)

Title CXCL9 and CXCL11 Chemokines Overproduced by Papillary Thyroid Cancer Cells, under the Influence of IFN- γ and TNF- α , Inhibit Proliferation and Migration, and Are Modulated by PPAR γ

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Body Until now, to our knowledge, no data are present in literature about the regulation by cytokines (IFN-gamma and TNF-alpha) of chemokines (C-X-C motif) ligand 9 and 11 (CXCL9 and CXCL11) in primary cell culture of papillary thyroid carcinoma (PTC), nor the effect of peroxisome proliferator-activated receptor-gamma (PPAR-gamma) activators on these chemokines, or the effect of these chemokines on PTC proliferation. The aims of this study were: a) to test the effect of IFN-gamma and TNF-alpha stimulation on the secretion of the CXC alpha-chemokines, CXCL9 and CXCL11, in primary cell cultures obtained from PTC, in comparison with normal thyroid tissue; b) to assess the effect of PPAR-gamma activation on CXCL9 and CXCL11 secretion and proliferation in these cell types; c) to evaluate the effect of CXCL9 and CXCL11 on proliferation and migration of PTC cells.

In normal thyroid cells and PTC cells, CXCL9 and CXCL11 were absent basally; a similar dose-dependent secretion of CXCL9 and CXCL11 was induced by IFN-gamma in both cell types. TNF-alpha alone induced a significant CXCL9 and CXCL11 secretion only in PTC cells. The stimulation with IFN-gamma+TNF-alpha induced a synergistic CXCL9 and CXCL11 release in normal cells; a higher secretion was induced in PTC cells. Treatment of normal cells with the thiazolidinediones suppressed dose-dependently IFN-gamma+TNF-alpha-induced CXCL9 and CXCL11 release, while stimulated CXCL9 and CXCL11 secretion in PTC cells. A significant antiproliferative effect by thiazolidinediones was observed only in PTC cells. CXCL9 or CXCL11 (500 pg/mL) induced a significant ($P<0.001$) antiproliferative effect in PTC cells and inhibit migration of PTC cells, too.

In conclusion, our study demonstrates that the treatment with IFN-gamma+TNF-alpha induces a marked release of CXCL9, and to a lesser extent of CXCL11, by primary cells from PTC. It has been shown a discrepancy between the stimulatory effect of thiazolidinediones on CXCL9 and CXCL11 secretion and the inhibitory role on PTCs proliferation. Furthermore, our study first shows that CXCL9 and CXCL11 are able to inhibit the proliferation and migration of primary PTC cells; the use of CXCL9 or CXCL11 as antineoplastic factors in PTC remains to be explored.

Nothing to Disclose: AA, SMF, SF, MM, AC, CM, PF, PM

Pub # P2-685

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Thyroid Cancer (1:30 PM-3:30 PM)

Title Differences in Intracellular and Extracellular miRNA Expression Profiles in Normal and Cancerous Thyroid Cells

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Body Recent analyses of miRNA profiles, indicative of tumors, in serum or other body fluids assume that the expression patterns of circulating miRNAs are similar to those in tumor cells. There is evidence that this assumption may not be true and that the mechanisms of miRNA release may yield different miRNA profiles in serum or body fluids compared to tumors. In this study, cell conditioned media served as an *in vitro* model to analyze miRNA release to body fluids. The aim was to compare miRNA expression profiles in thyroid cells to profiles of miRNAs released from cells to media. We hypothesized that these profiles would be different. Five cell lines were used, representing benign thyrocytes (ThJ) or thyroid cancer subtypes: papillary (LAM1), follicular (WRO, KAT1), and anaplastic (SW1736). The miRNA targets used here have been previously reported as relevant to thyroid cancer. MiRNA was extracted using Qiagen products: miRNeasy Kit for cells and QIAamp Circulating Nucleic Acid Kit for the media. Prior to miRNA extraction media was centrifuged to remove cell debris. The miScript RT Kit (Qiagen, Inc.) was used for cDNA synthesis and the miScript SYBR Green PCR Kit in combination with miRNA-specific primers was used to detect mature miRNAs. The control endogenous reference ncRNA snord25_1 was used to calculate $[\Delta]C_T$ values. ThJ miRNA expression was used to calculate normalized $[\Delta][\Delta]C_T$ values separately for each source of miRNA (media or cells), and the normalized media and cell miRNA expression profiles were compared. The results indicate that the expression profiles of the target miRNAs differ between the media and the cells. MiR-181a_1 in cells was up-regulated in LAM1, SW1736 and KAT1- fold changes respectively: 4.9, 3.1 and 10.7, but in the media it was highly down-regulated in LAM1, WRO and SW1736 (-27.5, -3.5 and -2.4). MiR-21-1 was up-regulated in LAM1, WRO, and KAT1 cells (2.1, 2.5, and 4.1), but down-regulated in the media of LAM1 by 2.8 and SW1736 by 2.5 fold. MiR-221_1 was up-regulated in LAM1, and KAT1 cells by 3.8 and 3 fold, but in the media no change was observed in KAT1. For miR-302c_1 there were no changes in thyroid cancer cells, whereas it was significantly down-regulated in their media. This suggests that miRNA profiles in cells do indeed differ from miRNA released to media and across thyroid cancer subtypes. A better understanding of these differences will be essential for development of clinically useful body fluid-based diagnostic tests.

Sources of Research Support: Vermont Cancer Center; College of Medicine, UVM, Internal Grant Program.

Nothing to Disclose: EIZ, WL, JHW, FEC

Pub # P2-686

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Thyroid Cancer (1:30 PM-3:30 PM)

Title Gene Expression Profile Using Microarray Analysis for Papillary Thyroid Carcinoma in a Patient with TSH-Secreting Pituitary Adenoma (TSHoma)

Author String D-J Lim, J-Y Sim, J-H Kim, J-H Han, M-I Kang, S-D Moon
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Body **Background:** Rare cases have been reported with both thyroid cancer and pituitary TSHoma but tumorigenesis and gene expression in thyroid under the influence of excessive TSH produced by pituitary adenoma were hardly known. The objective of our study was to investigate gene expression profile of papillary thyroid carcinoma (PTC) in a patient with pituitary TSHoma, using microarray analysis of both post operative PTC and normal tissues and to compare with sporadic PTC case.
Patients and Methods: PTC and adjacent normal thyroid tissues in pituitary TSHoma case and sporadic PTC case, respectively, which were gender-, age- and stage-matched, were analyzed using DASL (cDNA-mediated annealing, selection, extension and ligation) microarray assay. Microarray experiments were repeated and at least 4 fold change was considered to be significant results. For the selected genes showing significant fold change, real-time reverse transcription-polymerase chain reaction (RT-PCR) analysis was performed.
Results: After gene expression of both PTCs was adjusted by that of normal tissues (TSHoma-PTC tumor/normal vs. sporadic PTC tumor/normal), overexpressed 40 genes and underexpressed 138 genes in PTC with TSHoma were observed in microarray analysis, compared with sporadic PTC. Among these 178 genes, real-time RT-PCR was performed in selected 15 genes, showing that the expression of CASP4, CLDN10, FGF7, KLK11, LCN2, RELN, MMP12 and OTC was significantly different from that of sporadic PTC case.
Conclusions: Our data showed, for the first time, the unique gene expression profile in PTC with pituitary TSHoma, different from sporadic PTC. Our results suggest that TSH might contribute to the aggressiveness of PTC through the regulation of various genes.

Sources of Research Support: Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (5-2010-A0154-00044).

Nothing to Disclose: D-JL, J-YS, J-HK, J-HH, M-IK, S-DM

Pub # P2-687

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Hypothyroidism & Thyrotoxicosis (1:30 PM-3:30 PM)

Title Intact Parathyroid Hormone Measurement at 24 Hours after Thyroid Surgery: Predictive Value to Permanent Hypoparathyroidism

Author String T Julian, ML Granada, JM Balibrea, P Moreno, A Alastrue, M Puig-Domingo, A Lucas
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Body **Background.** There is very few information in the usefulness of postoperative intact parathyroid hormone determination (iPTH) to predict permanent hypoparathyroidism after thyroid surgery. The major aim of the present study is to determine the value of iPTH concentration after total thyroidectomy (TT) to predict permanent hypoparathyroidism. Secondary aims are to determine the prevalence of hypocalcemia (hypoCa) and iPTH deficiency after 24 h of this surgery, and its relationship with symptoms of hypoCa.

Methods. Forty-six consecutive patients who underwent TT between October 2009 and May 2010 were included in the study. iPTH (normal values 15-65 pg/mL, by an immunochemiluminometric technique; Modular analytic E170, Roche Diagnostic; functional sensitivity 6.0 pg/mL) and albumin-corrected serum calcium concentrations (Ca) were measured at 24 h, 1 and 6 months after surgery. HypoCa was defined as Ca < 2.10 mmol/L and iPTH was considered to be low if it was < 15 pg/mL, according to the low normal value of the assay. Permanent hypoparathyroidism was diagnosed in patients with serum iPTH < 15 pg/mL at 6 month after surgery. Symptoms of hypoCa were recorded.

Results. Twenty-nine (63 %) out of 46 patients presented hypoCa at 24 h after TT (7 of these 29 were symptomatic); 24 h post TT, iPTH was 26.1 ± 12.65 pg/mL vs 17.2 ± 12.5 pg/mL ($p < 0.001$) in normocalcemic vs hypocalcemic patients; 13 out of 46 (28.2 %) patients showed iPTH concentrations < 15 pg/mL. Twelve of them, developed hypoCa and 7, experienced symptoms. Patients with iPTH deficiency had lower postoperative Ca concentrations (1.97 ± 0.08 vs 2.08 ± 0.09 ; $p = 0.025$). Among the 29 patients showing postsurgical hypoCa, iPTH deficiency was detected in 4 (13.8%) at 1 month and in 3 (10.3 %) at 6 months. Among the 13 patients with low iPTH at 24 hours, iPTH deficiency was found in 5 (38.5 %) at 1 month and in 4 (30.8 %) at 6 months postsurgery. An iPTH concentration at 24 h postsurgery

Conclusions. An iPTH concentration > 5.8 pg/mL at 24 h after total thyroidectomy predicts normal parathyroid function at 6 months.

Disclosures: MP-D: Advisory Group Member, Merck BV. Nothing to Disclose: TJ, MLG, JMB, PM, AA, AL

Pub # P2-688

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Hypothyroidism & Thyrotoxicosis (1:30 PM-3:30 PM)

Title Gastrointestinal Malabsorption of Levothyroxine Sodium

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Body Convincing evidence support the need for an individually tailored dose of levothyroxine sodium. However, some patients fail to show adequate clinical and chemical response to the [ldquo]appropriate[rdquo] dose of T4. Hence, gastrointestinal disorders have been reported to reduce T4 absorption and, thus, its therapeutic efficacy. No systematic studies are available, however, on the clinical issues raised by levothyroxine malabsorption and this was the aim of our study. A total of 2018 sequentially examined adult patients (age:16-60 yrs) have been treated with an accurate schedule of T4 administration and followed up for at least 24 months. The expected serum TSH has been obtained in the [ldquo]responder[rdquo] patients at a median thyroxine dose of 1.33 [mu]g/kg/day in replacement modality (RM) and 1.56 [mu]g/kg/day in semi-suppressive modality (SM). However, 335 patients (17%) still failed to reach the expected serum TSH concentrations (0.5-2.0 mU/l in RM; 0.1-0.5 mU/l in SM). Once excluded the obvious causes of poor compliance and/or drug interference and the subjects still under investigation, 245 patients (12.1%; 222F/23M) were classified as [ldquo]non responder[rdquo]. These underwent a defined diagnostic algorithm for the presence of gastrointestinal disorders. A gastric disease has been diagnosed in 152/245 patients (H.pylori-related or autoimmune atrophic gastritis), while an intestinal disorder has been identified in 47/245 patients. Of these, 37 had upper intestinal disorders (celiac or parasitic disease, lactose intolerance) while 10 had chronic bowel inflammatory diseases (ulcerative colitis, Crohn disease, etc). In the remaining 46 patients, despite complete diagnostic workup, the cause of malabsorption remained unknown. The median required dose of T4, in patients with gastric disorders, was 1.73 [mu]g/kg/day (+30%) in RM and 1.91 [mu]g/kg/day (+22.4%) in SM, while in those with intestinal disorders was 1.84 [mu]g/kg/day (+38%) and 1.97 [mu]g/kg/day (+26%), respectively. Upon treatment of gastrointestinal disorders, the effect was reversed in 1/3 of patients with gastric diseases and in 2/3 of those with intestinal disorders. These data showed that: a) gastrointestinal malabsorption of oral thyroxine has been recognized in a significant number of treated patients; b) these gastrointestinal disorders account for most of T4 treatment failure and increase the cost for hormonal monitoring; c) in about half of these patients, T4 malabsorption was partially or totally reversible.

Nothing to Disclose: CV, SCDD, MGS, LG, MC

Pub # P2-689

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Hypothyroidism & Thyrotoxicosis (1:30 PM-3:30 PM)

Title Falsely Elevated Thyroid Hormone Levels Caused by a Substance Interfering with Ruthenium

Author String K Ohba, O Kimoto, K Iwahara, T Satoh, T Unno, A Matsushita, H Iwaki, H Matsunaga, S Suzuki, S Sasaki, Y Oki, H Nakamura
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Body Background: Some cases have been reported in which an endogenous substance has interfered with thyroid hormone measurement, resulting in falsely elevated FT4 and/or FT3 levels. We studied an interesting case showing a substance interfering with ruthenium (Ru).
Clinical Case: A 63-year-old woman consulted a nearby hospital because of diffuse goiter. Laboratory tests showed normal TSH (0.52 [mu]IU/L; normal range: 0.5-5.0) measured by sandwich electrochemiluminescent immunoassay (ECLIA, Elecsys; Roche, Basel, Switzerland), and elevated FT4 (3.8 ng/dL; normal range: 0.9-1.7) and FT3 (7.6 pg/mL; normal range: 2.3-4.3) measured by competitive ECLIA. After treatment with methimazole, she complained of general malaise and deterioration of the goiter. She was referred to our hospital for further evaluation. At that time, she showed normal FT4 but very high TSH. Methimazole was discontinued, and her symptoms improved one month later, but her thyroid hormone levels were elevated in spite of normal TSH. Since her past medical history was significant for rheumatoid arthritis, we considered the possible interference of some endogenous antibodies, and measured her thyroid hormones employing a chemiluminescence assay (CLIA, ADVIA Centaur; Siemens, Munich, Germany), which showed normal results (TSH: 1.1 [mu]IU/L, FT4: 1.3 ng/dL, and FT3: 3.1 pg/mL). We also found that the values for cortisol and estradiol measured by competitive ECLIA were higher than those measured by CLIA, whereas prolactin and insulin levels measured by sandwich ECLIA were lower than those by CLIA. Since Ru is a common component in ECLIA, we evaluated the patient's serum by modified ECLIA, in which Ru was replaced with sulfonated Ru to reduce the effect of the interfering substance. The results showed a marked reduction in FT4 and FT3. The possible mechanism is that some endogenous interfering substance reduces the excitation of Ru resulting in falsely higher or lower hormone levels. When the patient's serum was fractionated by HPLC gel filtration, there were two peaks of FT4 and three peaks of FT3. The retention time of the common peak was the same as that of IgA. The jacalin addition test which absorbs IgA showed a moderate reduction in FT4 and FT3, suggesting that the interfering substance is an IgA antibody.
Conclusion: This is the first case report demonstrating the presence of an endogenous IgA antibody against Ru, leading to falsely elevated thyroid hormone levels measured by ECLIA.

Nothing to Disclose: KO, OK, KI, TS, TU, AM, HI, HM, SS, SS, YO, HN

Pub # P2-690

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Hypothyroidism & Thyrotoxicosis (1:30 PM-3:30 PM)

Title A Case of Thyroid Storm Following RAI Treatment Managed Successfully with Plasma Exchange

Author String N Malhotra, SC Kumar, KH Hupart
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Body

Background:
Thyroid storm is an acute life threatening complication of hyperthyroidism. It has been uncommonly reported following the use of I-131 treatment. We report the case of a patient with Graves' Disease, previously treated with Methimazole, who was administered I-131 and developed thyroid storm. He was subsequently managed with multiple modalities including lithium, oral bile acid sequestrants and plasma exchange.

Case:
49 year old man treated with Methimazole for 3 years. Despite Methimazole being administered at a nursing home, he remained biochemically and clinically thyrotoxic. His medical history was significant for HIV/AIDS, Hepatitis C, COPD, HTN, CVA and Seizures. Prior to therapy, an I-123 thyroid uptake was 74.6% and homogenous. He received 10.3 mCi I-131. His condition deteriorated by day 3 of treatment. He became delirious with a BP: 170/100 mm Hg, Temp: 103[deg]F, HR: 144 bpm typifying thyroid storm. He was medicated with intravenous Esmolol, Methimazole, Hydrocortisone, oral bile acid sequestrants and required medical intensive care. Subsequently, on Day 4 the patient was intubated and vasopressors were required; SSKI and Lithium carbonate were added. He underwent plasmapheresis and plasma exchange giving him 3L of fresh frozen plasma. This was repeated on day 5. His HR improved from 150 bpm on day 3 to 100 bpm on day 7, T4 from >24 to 12.2 mcg/dl, T3 from 609 to 173 ng/dl. The patient continued to receive aggressive supportive care and fully recovered within 2 weeks.

Discussion:
Thyroid storm is a rare complication of I-131 therapy. Our patient received Methimazole prior to I-131 and was treated with Lithium, SSKI, Hydrocortisone, bile acid sequestrants and plasma exchange. However, the marked changes that were seen in laboratory data and in the timing of his clinical response suggest that plasma exchange proved markedly beneficial in this case. The goal of our treatment was to block the synthesis of thyroxine with Methimazole, block its release with SSKI and Lithium, block its conversion to T3 with glucocorticoids, increase its clearance initially with bile acid sequestrants and then with plasmapheresis and to decrease the free fraction of circulating thyroid hormone by plasma exchange by giving the patient additional thyroid hormone binding sites.

Conclusion:
This aggressive approach targeting multiple aspects of thyroid hormone metabolism proved successful and we will portray the clinical details and timecourse of his recovery.

Nothing to Disclose: NM, SCK, KHH

Pub # P2-691

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Hypothyroidism & Thyrotoxicosis (1:30 PM-3:30 PM)

Title Combination of Recombinant TSH (rhTSH) and Lithium Overcomes Amiodarone-Induced Low Radioiodine Uptake in a Thyrotoxic Female

Author String NER Laplano, LM Asis
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Body INTRODUCTION: rHTSH increases RAIU in selected populations, while lithium is used as adjunct to RAI therapy in Graves' disease with low RAIU. In this report, both drugs when combined overcame the low ¹³¹Iodine uptake in a Graves' patient.
CLINICAL CASE: A 39-year old female with Graves' disease acquired thionamide-induced agranulocytosis and severe hypokalemia, and subsequently went into cardiorespiratory arrest. On resuscitation, she had ventricular tachyarrhythmias which were cardioverted using amiodarone. She was subsequently placed on i.v. hydrocortisone (50mg every 8h), p.o. amiodarone (200mg/day) and propranolol (20mg every 8h). Once hemodynamically stable, she was transferred to our institution for definitive Graves' disease management. On admission, she was normotensive, tachycardic, and afebrile. She had fine tremors, hyperreflexia, with diffuse, non-tender thyromegaly. Initial investigation showed normal CBC, hypokalemia (3.51mmol/L, NV 3.7-5.0), and elevated ALT (40.76 U/L, NV up to 31). TSH was low (0.03 uIU/L, NV 0.27-3.75). Thyroid ultrasound showed diffuse thyromegaly (right: 5.2 x 1.3 x 1.7cm; left: 5.2 x 1.4 x 1.8cm) with uniform echopattern and normal color flow doppler. After 2MBq ¹³¹I, RAIU showed low uptake at 4th hour and 24th hour (6% and 7%, respectively). In preparation for RAI therapy, she was given lithium 900mg/day for 12 days to increase RAI retention. To increase ¹³¹Iodine uptake, two doses of 0.9mg rhTSH were injected intramuscular 24 hours apart and two days before RAI therapy. Repeat RAIU after the 2nd dose of rhTSH showed a more than 5-fold increase in 4th hr uptake compared from baseline (32% vs. 6%). Exactly 24 hours after the 2nd dose of rhSTH, she was given ¹³¹I 25mCi. Clinical and biochemical course continuously improved thereafter. Presently she is back to work as a grade school teacher.
CONCLUSION: This is the first case to demonstrate the possible efficacy of combining rhTSH and lithium to overcome amiodarone-induced low ¹³¹Iodine uptake in Graves' disease.

Nothing to Disclose: NERL, LMA

Pub # P2-692

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Hypothyroidism & Thyrotoxicosis (1:30 PM-3:30 PM)

Title A Euthyroid Woman with Macro-TSH

Author String A Gessl, S Blueml, C Porzig, W Stock, C Bieglmayer
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Body A 68 year old woman was referred for regular care to the outpatient clinic of Rheumatology of our hospital. She received methotrexate for her rheumatoid arthritis and was otherwise healthy. As routine lab workup TSH was determined and yielded a value of 170.80 mU/L, free T4 was 1.27 ng/dL, free T3 3.03 pg/mL, total T3, T4 and TSH-receptor antibodies were also in the normal range. She had no signs or symptoms of hypothyroidism. SHBG was slightly elevated (79.1 nmol/L), but TBG was in the high normal range (29.3 mg/L). Her own, as well as her family[acute]s thyroid history were completely uneventful. A short course of thyroxine was not tolerated. Thus, (subclinical) hypothyroidism with grossly elevated TSH was unlikely. TSH was repeatedly elevated in various different assays and blocking reagents directed to block heterophilic antibodies as well as serial dilutions showed no change in TSH concentration. Finally, polyethylene glycol (PEG) precipitation was performed and yielded a TSH value of 3.88 mU/L (recovery 2%). Recovery for LH was 60%, for FSH 103%, for β HCG 81%. Thus, an autoantibody against TSH (in analogy to Macro-PRL) was suspected, which shows slight crossreaction with LH. Further studies are currently under way to substantiate this extremely rare finding.

Disclosures: CP: Employee, Roche Diagnostics. WS: Employee, Roche Diagnostics. Nothing to Disclose: AG, SB, CB

Pub # P2-693

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Hypothyroidism & Thyrotoxicosis (1:30 PM-3:30 PM)

Title Thyroid Hormone Resistance and Pituitary Incidentaloma

Author String LA Caetano, DY Eimori, RD Severino, ACW Xavier, APT Liberati, SMP Vecchiatti, SR Correa-Silva, RA Guerra
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Body **BACKGROUND:** Resistance to Thyroid Hormone (RTH) is a rare syndrome, characterized by persistently elevated levels of thyroid hormones (TH) and normal or high TSH. This disease occurs due to mutations in the beta-isoform of the thyroid hormone receptor (TR- β) gene in about 90% of cases. Consequently there is a defect in the TH receptor, which leads to an insensitivity of tissues to the action of TH. The phenotype is highly variable even with identical mutations of TR- β . The main differential diagnosis is a TSH-secreting pituitary tumor. Currently, there are over 700 reported cases of RTH, but to our knowledge, in the literature, there are descriptions of only four cases of pituitary adenoma in the setting of documented RTH.
CLINICAL CASE: A 58-year-old postmenopausal woman, with prior hypertension, was referred to our Unit because of changes in thyroid function found during routine tests. She only complained of anxiety. On physical examination she had HR=68bpm, BP=130x80mmHg, slightly enlarged thyroid without palpable nodules; absence of lid lag or exophthalmos or tremor. Laboratory tests showed TSH=6.76mU/L (RV: 0.4-4.0), FT4=2.99ng/dL (0.89-1.76), undetectable anti-peroxidase and anti-thyroglobulin. During follow up, levels of FT4 and TSH persisted elevated. Thyroidal ¹³¹I uptake at 24 hr was 46%. She had a normal TSH response to TRH (basal TSH = 5.04; peak level at 15 minutes = 14.0), alpha-glycoprotein subunit level was 1,040ng/L (340-4,000) and SHBG was 69.2nmol/L (18-114). These data suggest that inappropriate TSH secretion in the present patient resulted from Resistance to Thyroid Hormone. During the investigation sellar MRI was performed and a pituitary adenoma of 0.6x0.4cm was seen. All pituitary hormones were normal. As the patient had no clinical signs of hyperthyroidism, it was chosen by observation without any specific medication. The patient remains clinically stable so far (follow up of 4 years).
CONCLUSION: This case represents a rare presentation of RTH coexisting with an incidental pituitary adenoma. As a pituitary incidentaloma can be present in 10-20% of the general population, it can also occur in patients with RTH. Moreover, the question of whether RTH could lead to pituitary hyperplasia or adenoma development was also raised in the literature. Therefore, careful hormonal evaluation prior to sellar MRI is necessary in order to do the correct differential diagnosis in a case of hyperthyrotropinemia associated with hyperthyroxinemia.

Nothing to Disclose: LAC, DYE, RDS, ACWX, APTL, SMPV, SRC-S, RAG

Pub # P2-694

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Hypothyroidism & Thyrotoxicosis (1:30 PM-3:30 PM)

Title The Association between Thyroid Function and the Prevalence and Incidence of the Metabolic Syndrome in Older Adults

Author String AC Waring, N Rodondi, S Litwack-Harrison, A Kanaya, E Simonsick, I Miljkovic, S Satterfield, A Newman, DC Bauer
UCSF, San Francisco, CA; University of Lausanne, Lausanne, Switzerland; California Pacific Medical Center Research Institute, San Francisco, CA; Johns Hopkins University, Baltimore, MD; University of Pittsburgh, Pittsburgh, PA; University of Tennessee Health Science Center, Memphis, TN; UCSF, San Francisco, CA

Body **Context:** Both subclinical hypothyroidism and metabolic syndrome (MetS) have been associated with increased coronary heart disease events in older adults. It is unknown if the prevalence and incidence of MetS is increased in individuals with subclinical thyroid dysfunction.
Objective: To examine the relationship between levels of subclinical thyroid dysfunction and the prevalence and incidence of MetS in a cohort of older adults.
Design, Setting and Participants: Health ABC is a prospective cohort of 3075 community-dwelling men and women aged 70-79 years at enrollment. Participants were recruited from 2 US cities (Pittsburgh, PA and Memphis, TN) between 1997-1998 and followed yearly. TSH was measured on 2799 members of the cohort. Participants missing TSH values, with overt hyperthyroidism, diabetes at baseline, or taking anti-thyroid medications, lithium, or amiodarone were excluded, leaving a sample of 2156.
Measurement and Analysis: TSH was measured using a third-generation immunoassay. Free thyroxine was measured in participants with a TSH<0.1 or >7mIU/L. Categories of thyroid function were defined as subclinical hyperthyroid, euthyroid, mild (TSH 4.5-9.9) and marked (TSH 10-20) subclinical hypothyroid, an overt hypothyroid. MetS, defined per revised ATP III criteria, was assessed at baseline and after 6 years of follow-up. The relationship between thyroid function category and prevalent and incident MetS was analyzed using logistic regression adjusted for age, gender, and race.
Results: At baseline, 684 participants met criteria for MetS (31.7%). MetS was significantly more common in women (p<0.01) and white (p<0.01) participants. Subclinical hypothyroidism with TSH 10-20 (n=34) was associated with an increased odds of MetS at baseline (OR 2.2, 95% CI 1.1-4.4). No association between other categories of thyroid function and MetS was found. Incident MetS developed in 26.1% of women and 20% of men (239 incident cases). The odds ratio for incident MetS in participants with subclinical hypothyroidism with TSH 10-20 (n=12) was similar to the odds of prevalent MetS in this group (OR 2.4, 95% CI 0.7-7.6), however the confidence interval was wide, perhaps due to a small number of events. Results were unchanged after adjustment for BMI, smoking, and HOMA-IR.
Conclusion: Subclinical hypothyroidism with a TSH >10 is associated with an increased prevalence of metabolic syndrome in older adults which may contribute to increased cardiovascular morbidity and mortality

Sources of Research Support: Health ABC is supported by the National Institutes of Aging (NIH).

Nothing to Disclose: ACW, NR, SL-H, AK, ES, IM, SS, AN, DCB

Pub # P2-695

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Hypothyroidism & Thyrotoxicosis (1:30 PM-3:30 PM)

Title Resistant Thyrotoxicosis with Concomitant Pulmonary Sarcoidosis

Author String U Yanamandra, N Kotwal, AS Menon, A Ahluwalia, V Nair
Armed Forces Medical College, Pune, India

Body
Background: Autoimmune endocrinopathies and less commonly, thyroid autoimmune disease have been reported in patients with sarcoidosis. Similarities in the pathogenesis of Graves' disease and sarcoidosis may explain the prevalence of thyroid autoimmunity in patients with sarcoidosis, even in the absence of thyroid involvement. Direct involvement of the thyroid gland with sarcoidosis is rare. Postmortem studies indicate that the thyroid gland is involved in 4.2 - 4.6% of patients with sarcoidosis. [1, 2] The initial clinical manifestation in cases of sarcoid involvement of the thyroid gland may be hypothyroidism, thyroiditis, or Graves' disease.
Clinical Case: We report a case of Graves' disease with concomitant pulmonary sarcoidosis. This 37-year-old man presented with clinical symptoms suggestive of thyrotoxicosis. Laboratory studies (T₃ 2.58ng/ml, T₄ 21.41mcg/dl, TSH 0.02mIU/L) and findings on thyroid uptake scan (Technitium uptake - 6.6%) were consistent with Graves' disease. He was also found to have hilar lymphadenopathy. He did not achieve remission inspite of being on antithyroid drugs for two years and having received two sessions of radioiodine therapy (29 mCi of ¹³¹I and 26 mCi of ¹³¹I given 08 months later). Repeat thyroid profile revealed thyrotoxicosis (T₃ 1.82ng/ml, T₄ 22.9 mcg/dl, TSH 0.03mIU/L). Fiberoptic bronchoscopy and transbronchial lung biopsy from right lower lobe - superior segment was done. Histopathological examination revealed noncaseating granulomas consistent with sarcoidosis. Serum ACE was 106 IU/L (normal < 60 IU/L). Fine needle aspiration of the thyroid gland revealed ill formed elements of granulomatous tissue. Biopsy could not be done due to unwillingness of patient. A provisional diagnosis of sarcoidosis of thyroid was made. The patient responded well to steroids (prednisolone 1mg/kg) with complete resolution of thyrotoxic symptoms and pulmonary lesions. This therapeutic resolution further reiterates the diagnosis of sarcoidosis of thyroid which requires biopsy for firm diagnosis.
Conclusion: Thyroid sarcoidosis should be considered in a patient of thyrotoxicosis with concomitant pulmonary sarcoidosis not responding to antithyroid drugs/ radioiodine therapy. Sarcoidosis of the thyroid may lead to an unusual or suboptimal clinical response of Graves' disease to different treatment modalities, such as in our current patient.

1. Yarman S, Kahraman H, Tanakol R, Kapran Y. Concomitant association of thyroid sarcoidosis and Graves' disease. *Horm Res.* 2003;59:43-46.
2. Vailati A, Marena C, Aristia L, et al. Sarcoidosis of the thyroid: report of a case and a review of the literature. *Sarcoidosis.* 1993;10:66-68.

Nothing to Disclose: UY, NK, ASM, AA, VN

Pub # P2-696

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Hypothyroidism & Thyrotoxicosis (1:30 PM-3:30 PM)

Title Euthyroid Hypothyroxinemia Associated with Nonimmune Hydrops Fetalis

Author String AD Briscoe, ME Gottschalk
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Body
Background: Nonimmune hydrops fetalis (NIHF) presents with abnormal fluid accumulation in 2 or more body compartments, including: ascites, pleural effusion, pericardial effusion and skin edema. Prior case reports have described NIHF in association with congenital hypothyroidism, primary hypothyroidism, central hypothyroidism, transient hypothyroidism from maternal Graves disease treated with Propylthiouracil, and hyperthyroidism. Autopsy reports of cases of NIHF find microscopic changes of endocrine organs most consistently, including thyroid hyperplasia. We report the first case of deficiency of all 3 thyroid hormone binding proteins (THBP) in a euthyroid infant associated with NIHF.
Case: A 34week premature male infant with NIHF including skin edema and bilateral pleural effusions, had thyroid function tests obtained per NICU protocol at 1 month of age. The CA newborn screen was negative for congenital hypothyroidism (TSH 0.43[mu]IU/mL) at 52hr of life. At the 1mo screen the T4 was 1.9mcg/dL (7.2-15.2), TSH 1.4[mu]IU/mL (0.35-5), and free thyroxine (fT4) 0.4ng/dL (0.71-1.85). The infant had no signs of hypothyroidism, no recent iodine use, and there was no family history of thyroid disease. He had hypoproteinemia due to NIHF with a total protein of 4.9g/dL (5.4-7), and albumin 1.7-3.4g/dL (2.6-3.6). He had required multiple albumin infusions with the most recent infusion completed on the day that T4 and TSH were drawn (albumin 3.4). On the day fT4 was drawn, the albumin was 2.9. To evaluate the abnormal thyroid tests, T3 uptake (T3U), thyroxine binding globulin (TBG), fT4 by dialysis and transthyretin (TTR) were obtained. fT4 by dialysis was normal, 2.0ng/dL (0.8-2) indicating that the routine hospital platform assay was affected by THBPs. Normal fT4, low T4 and a normal TSH is indicative of THBP deficiencies. T3U was 55.1% (25-35) and TBG was 6.8mcg/mL (12-26), TTR was 16mg/dL (19-38) and albumin was low indicating combined TBG, TTR and albumin deficiencies.
Conclusion: The infant did not have a congenital thyroid disorder, although he did exhibit all 3 THBP deficiencies in association with NIHF. Infants with NIHF should be screened for permanent thyroid disease as well as transient thyroid disease such as THBP deficiency given their hypoproteinemic state so that they receive replacement therapy if required. Once the hydrops resolves, they should be retested for THBP deficiencies to evaluate for a permanent deficiency.

Sources of Research Support: The fellow is supported by a fellowship training grant from Genentech Center for Clinical Research in Endocrinology.

Nothing to Disclose: ADB, MEG

Pub # P2-697

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Hypothyroidism & Thyrotoxicosis (1:30 PM-3:30 PM)

Title A Patient with Pendred Syndrome Showing Hyperthyroidism -- No Need for Perchlorate Discharge Test to Diagnose Pendred Syndrome

Author String S Fukata, A Hishinuma, N Nakatake, J Tajiri
Tajiri Clinic, Kumamoto, Japan; Dokkyo Medical University, Tochigi, Japan

Body [Background]
Pendred syndrome (PS; OMIM 274600) is an autosomal recessive disorder characterized by sensorineural deafness, goiter, and a partial defect in iodine organization. The size of goiters ranges from no enlargement of the thyroid gland to huge goiters. Despite the presence of a partial defect in iodine organization, patients with PS show normal thyroid function, especially in countries with a high iodide intake. Herein, we report a very rare case of PS with hyperthyroidism.

[Case]
A 39-yr-old woman attended our clinic with weight loss and general malaise in October, 2007. She had severe hearing loss from birth. Thyroid function tests revealed subclinical hyperthyroidism; TSH 0.047 [μ]IU/ml (normal: 0.220-3.30), FT4 1.38 ng/dl (0.9-1.8), FT3 3.34 pg/ml (2.2-4.3), Tg 89.6 ng/ml (<35), anti-TgAb 10.0 IU/mL (<28), anti-TPOAb 5.6 IU/mL (<16), TBII 7.4% (<20) and RAIU (1 hour) 9.34%. Ultrasonography showed a diffuse goiter with estimated volume of 64.5 ml. Although her thyroid dysfunction was very mild, because of the existence of symptoms we initiated treatment with iodide potassium. However, FT4 did not decrease, but increased to 2.13 ng/dl. Therefore, we treated her with radioisotope therapy twice. Consequently, thyroid function had normalized 1 year after the first visit to our clinic. Also, the volume of goiter decreased to 40 ml. Because at this point the possibility that the patient had PS could not be excluded, we performed CT scan of the petrous bones to see whether enlargement of the vestibular aqueduct (EVA) was present or not. As a result, bilateral EVA was shown clearly. Hence, PS was strongly suspected and we analyzed the PDS gene, in which mutation of p.His723Arg (homozygous) was demonstrated. Finally the diagnosis of PS was confirmed.

[Conclusion]
To our knowledge, there are only two reported cases of PS showing hyperthyroidism. Although perchlorate discharge test is said to be useful for the diagnosis of PS, considering the presence of these cases of PS with hyperthyroidism, we recommend the following criteria to diagnose PS: 1) The presence of congenital sensorineural hearing loss. 2) Regardless of the presence of goiter or the degree of thyroid dysfunction including hyperthyroidism, CT scan or MRI should be performed to demonstrate bilateral EVA. 3) If EVA is detected, the PDS gene should be analyzed. The perchlorate discharge test is not necessary to diagnose PS.

Nothing to Disclose: SF, AH, NN, JT

Pub # P2-698

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Hypothyroidism & Thyrotoxicosis (1:30 PM-3:30 PM)

Title An Old Tool for an Effective Diagnosis of Thyroid Autonomy

Author String F Ianni, G Perotti, RM Paragliola, MP Ricciato, L Castellino, A Prete, V Luotto, A Pontecorvi, SM Corsello Catholic University School of Medicine, Rome, Italy; Catholic University School of Medicine, Rome, Italy

Body Recent guidelines for the evaluation of thyroid nodules recommend scintigraphy (TS) only in case of low TSH value. In our daily clinical practice we observe that many patients (pts) with autonomous functioning thyroid nodules (AFTN), particularly in iodine deficient areas, do not have abnormal TSH value. The aim of this study was to evaluate clinical and imaging findings in these cases, in order to determine their predictive value. We retrospectively analyzed 381 TS performed in our institution between September 2009 and October 2010. Seventy-eight pts (68F and 10M, mean age 65.8 yrs, range 33-82) presented at least one autonomous nodule. All underwent clinical examination, TSH, FT3, FT4 and TPOAb determinations, 24h radioiodine uptake (RAIU) and Doppler US; uninodular goiter (UG with AFTN) was found in 24 pts and multinodular goiter (MG with AFTN) in 54. Patients were divided into two groups on the basis of TSH values above (group 1) and below (group 2) 0.35 mIU/ml respectively. Group 1 (25 pts; 11 UG, 14 MG): laboratory results showed TSH 0.82 ± 0.42 mIU/ml, FT3 3.09 ± 0.68 pg/ml, FT4 8.14 ± 4.66 pg/ml (mean \pm SD); mean nodule diameter was 19.8 ± 9.4 mm; eleven nodules were isoechoic, two hyperechoic, and twelve hypoechoic. Doppler US pattern was type I in four, type II in six, and type III in fifteen pts. TS showed partial inhibition in 18 pts and total inhibition in 7 pts. The 24h RAIU was 27.7% in UG and 19.5% in MG respectively. Group 2 (53 pts; 13 UG, 40 MG): TSH 0.15 ± 0.08 mIU/l, FT3 4.2 ± 1.6 pg/ml, FT4 12.9 ± 3.1 pg/ml. The mean nodule diameter was 28.6 ± 14.2 mm; 27 nodules were isoechoic, nine hyperechoic, and 17 hypoechoic. Doppler US pattern was type I in 14 pts, type II in 15, and type III in 24. TS showed partial inhibition in 40 pts and total inhibition in 13 pts. The 24h RAIU was 29.6% in UG and 20% in MG, respectively. Statistical analysis demonstrated a significant difference between the groups only regarding FT3 ($p<0.01$) and FT4 ($p<0.01$), and RAIU in UG subgroup ($p<0.05$). We observed inverse correlation between the nodule diameter and TSH levels and in all pts a prevalent isoechoic pattern with peri-intranodular vascularization. In conclusion our results suggest that the use of TS in selected patients (subclinical hyperthyroidism, clinical signs and/or symptoms suspicious for hyperthyroidism, and finding of follicular lesions at FNAB), even with TSH still within reference range can detect AFTN.

Nothing to Disclose: FI, GP, RMP, MPR, LC, AP, VL, AP, SMC

Pub # P2-699

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Hypothyroidism & Thyrotoxicosis (1:30 PM-3:30 PM)

Title Coronary Vasospasm Mimicking Diffuse Coronary Artery Disease in Thyroid Storm

Author String C Fratianni, NE Speil, C Mamillapalli, E Rico, R Khardori
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Body While isolated coronary artery spasm has been well described among the cardiovascular manifestations of hyperthyroidism, a diffuse coronary blood flow abnormality suggestive of diffuse coronary artery disease (CAD) has not been reported prior. We report the case of a patient with thyroid storm whose angiographic findings mimicked multiple vessel CAD.

A 44-year-old previously healthy woman was admitted to the hospital with acute respiratory failure due to pulmonary edema that required mechanical ventilation. She had had 3 months of dyspnea, [ldquo]hot flushes [rdquo], profound fatigue, accompanied by a 60-pound weight loss.

On presentation she had sinus tachycardia 180 beats/min, blood pressure of 205/123 mm Hg, fever of 39.1 C, unremarkable electrocardiogram and troponin I 0.18 ng/ml (indeterminate range). Her physical exam showed goiter, signs of thyrotoxicosis and onycholysis of distal nail beds. Echocardiogram revealed mild global hypokinesis with depressed ejection fraction of 30-35% and cardiac catheterization was suggestive of severe CAD with multivessel 70% stenosis. Cardiac output was high at 12 L/min (normal range 2.5 to 4.0 L/min per m²)

Thyroid function tests obtained prior to cardiac catheterization confirmed hyperthyroidism: TSH 0.02 IU/ml, thyroxine (T4) 26.3 mcg/dl (6.1-12.2), free T4 3.9 ng/dl (0.6-1.6), total T3 228 ng/dl (87-178), T3 uptake 52% (32-48). TBII 90% (<10) and TSI 135% (<130) were consistent with Graves' disease. Based on clinical criteria she was diagnosed with thyroid storm and treatment with high dose propylthiouracil, lithium, glucocorticoids and beta-blockers was initiated with rapid symptomatic improvement. Within four days her cardiac ejection fraction dramatically improved to normal (50-60%). After 3 months of thionamide therapy she became biochemically euthyroid and a repeat cardiac catheterization showed normal coronary anatomy.

This case demonstrates the presence of severe coronary artery vasospasm with left ventricular LV dysfunction in a patient during thyroid storm and argues in favor of a cautious interpretation of any angiographic findings in this scenario. The rapid reversibility of both the LV dysfunction and the angiographic abnormalities suggests a role for temporary dysfunctional changes in coronary arteries, an important entity to be recognized in hyperthyroidism since this pathology can be ameliorated with treatment of the underlying thyroid disease, hence avoiding unnecessary surgical interventions.

Nothing to Disclose: CF, NES, CM, ER, RK

Pub # P2-700

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Hypothyroidism & Thyrotoxicosis (1:30 PM-3:30 PM)

Title Radioiodine Therapy in Elderly Patients with Nontoxic Multinodular Goiter and High Cardiovascular Risk: A Safe Therapeutic Option?

Author String RA Oliveira, PA Medeiros, LS Caminha, ES Pinto, PB Araujo, NA Silva, RG Souza, AP Lazaro, RL Zagury, FL Conceicao, RC Mello, M Vaisman
University Hospital Clementino Fraga Filho - Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

Body INTRODUCTION: Surgery is the best treatment of nontoxic multinodular goiter which is more prevalent in the elderly who often have prohibitive surgical risk. Radioiodine (¹³¹I) therapy has as a drawback the fact that NTMG usually accompanies a low thyroid ¹³¹I uptake (RAIU). The introduction of recombinant human TSH (rhTSH - Thyrogen[reg]) allows an increased thyroid RAIU and radiation absorbed by the thyroid after ¹³¹I therapy and therefore a more efficient reduction of the goiter volume. Data on this treatment are still scarce among the elderly population.

OBJECTIVE: To evaluate the safety of the ¹³¹I therapy after administration of rhTSH in the treatment of NTMG in elderly patients with contraindication to surgery.

PATIENTS AND METHODS: A prospective study with four elderly with high cardiovascular risk and prohibitive surgical risk and indication of treatment for NTMG: intrathoracic goiter (IG) and/or compressive symptoms. Patient 1: 85-y woman, extensive IG, compressive symptoms, arterial hypertension (AH), obesity, atrioventricular block with permanent pacemaker; Patient 2: 85-y woman, IG, AH, dyslipidemia; Patient 3: 63-y man, IG, compressive symptoms, severe coronary heart disease (CHD) and heart failure; Patient 4: 84-y man, IG, compressive symptoms, CHD, diabetes mellitus type 2 and AH. All patients were submitted to scintigraphy and thyroid RAIU 4 hours after administration of 40 microCi of Na¹³¹I at D1. At D2, 0,2 mg of rhTSH were administered. After 24 hours (D3), new scintigraphy I¹³¹I uptake was performed, followed by therapeutic dose of 29 mCi of Na¹³¹I. The patients remained hospitalized during the entire procedure, having been discharged 3 days after the therapeutic dose.

RESULTS: All patients exhibited a homogeneous and significant increase of the thyroid RAIU 24 hours after the administration of rhTSH: Patient 1: 2,34%[rarr]20,37%; Patient 2: 3,97%[rarr]31,58%; Patient 3: 1,96% [rarr]44,15%; Patient 4: 2,65%[rarr]30,93%. The patients did not have important clinical complications, with only patient 3 having had mild and self-limited dyspnea at the day of the therapeutic dose of ¹³¹I. All patients were reevaluated as outpatients one week after the procedure, with no complications in this period.

CONCLUSION: The treatment of NTMG with ¹³¹I preceded by rhTSH seems to be a safe therapeutic option in the elderly with high cardiovascular risk. However, a higher number of patients must be studied, as well as its long-term results.

Nothing to Disclose: RAO, PAM, LSC, ESP, PBA, NAS, RGS, APL, RLZ, FLC, RCM, MV

Pub # P2-701

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Hypothyroidism & Thyrotoxicosis (1:30 PM-3:30 PM)

Title Various Etiologies of Thyrotoxicosis Diagnosed in a General Medicine Clinic of University Hospital

Author String H Saito, N Saito, K Nomura
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Body
Purpose Graves' disease (GD) is a major cause of thyrotoxicosis. Other causes of thyrotoxicosis includes subacute thyroiditis (SAT), painless thyroiditis (PT), autonomously functioning thyroid nodule (AFTN) among others. The symptoms are often transient, non-specific or mild, and therefore, could be dismissed in the primary clinic. In order to examine the difference on thyrotoxicosis patients between a primary clinic and a tertiary one, we compared the profiles of patients in the department of general medicine (GM) and of endocrinology (ENDO) in the same university hospital.
Methods Fifty-eight consecutive cases of thyrotoxicosis diagnosed in the GM clinic during the past five years and 104 cases in the ENDO during one year were analyzed. Diagnosis was made by board-certified Endocrinologists not only in the ENDO clinic but also in the GM clinic. Their gender, age, symptoms and signs, etiologies of thyrotoxicosis and hormone levels were evaluated.
Results Difference was observed at the prevalence of rare disease etiologies. Rare diseases such as AFTN, TSH-secreting pituitary adenoma (TSHoma), and exogenous thyrotoxicosis appeared more frequent in a GM clinic than an ENDO clinic (AFTN 3.4% (n=2) vs. 1.0% (n=1), TSHoma 1.7% (n=1) vs. 0%, exogenous thyrotoxicosis due to weight-loss drug 3.4% (n=2) vs. 0%). Concerning more popular diseases such as GD, SAT and PT, no difference in prevalence were observed between the clinics (GD at GM and ENDO, 58.6% (n=34) vs. 65.4% (n=68), respectively, SAT 10.3% (n=6) vs 3.9% (n=4) and PT 19.0% (n=11) vs 17.3% (n=18). Gestational transient thyrotoxicosis (n=4) and amiodarone-induced thyrotoxicosis II (n=6) were referred to the ENDO clinic after screening in an OB/GYN and cardiology clinics, and were not diagnosed in a GM clinic. Other profiles were not different between the clinics.
Conclusion Present study suggests that prevalence of thyrotoxicosis due to AFTN, TSHoma and exogenous agent is rare in the tertiary clinic; however these are disease conditions that should not be disregarded in a primary clinic.

Nothing to Disclose: HS, NS, KN

Pub # P2-702

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Hypothyroidism & Thyrotoxicosis (1:30 PM-3:30 PM)

Title The Endocrine and Metabolic Profile in Adult Patients with Down Syndrome

Author String MD Musat, L Zah, R Danciulescu, D Aflorei, C Giurca, C Poiana
Carol Davila University of Medicine and Pharmacy, Bucharest, Romania; C I Parhon National Institute of Endocrinology, Bucharest, Romania

Body

Introduction: Recent data acknowledges prevention and adequate survey of the co morbidities associated with Down syndrome are ought to be done from early stages, in order to increase life expectancy and the quality of life for these individuals.

Objective: In Romania, there are no epidemiological data regarding the incidence and the impact of endocrine disorders in patients with Down syndrome. In this matter we have assessed the endocrine-metabolic parameters in a cohort of 18 young adults with Down syndrome in a retrospective observational study.

Material and Method : 11 males and 7 females aged 16-35, diagnosed with Down syndrome from their childhood were assessed for metabolic and endocrine profile in the Thyroid Department of the C.I.Parhon National Institute of Endocrinology between Jan 1st and Nov 30th 2009.

Results: Twelve patients were overweight or obese and 5 patients had dyslipidemia. Eight patients had hypothyroidism 9 were euthyroid and 1 had subclinical hyperthyroidism. A positive correlation was found between hypothyroidism and hypertriglyceridemia (Spearman rank correlation test $p=0.0112$). One male patient had impaired glucose tolerance test. Two patients one male and one female had hypogonadism. Fourteen patients had low stature, and 5 of them had normal IGF1.

Discussion: The patients in the studied cohort were members of Down Syndrome Association Bucharest, who are involved in a program of social integration by physical education and occupational therapies, which may influence their metabolic status. Subclinical hypothyroidism was the most frequent endocrine disorder in the studied group. Growth retardation, probably, is not due to a deficiency of growth hormone, but more studies need to be done.

Conclusion: The most common endocrine disorders in Down syndrome are thyroid dysfunctions, which may be obscure and should be actively sought in these patients. The most frequent metabolic disorders is dyslipidemia. The thyroid replacement should be introduced early in patients with Down syndrome and hypothyroidism in order to improve their metabolic and endocrine status and prevent cardiovascular complications. Physical activity also improves glucose and lipid metabolism in patients with Down syndrome

Nothing to Disclose: MDM, LZ, RD, DA, CG, CP

Pub # P2-703

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Hypothyroidism & Thyrotoxicosis (1:30 PM-3:30 PM)

Title Benign Transient Hyperthyroxinemia Following Surgical Neck Exploration: A Report on Three Cases

Author String E Rico, N Speil, C Mamillapalli, S Gupta, R Khardori
Southern Illinois University School of Medicine, Springfield, IL

Body
Background: Transient changes in thyroid hormone levels following surgical neck exploration have been sporadically reported. This phenomenon is often overlooked due to its rarity. We report three cases encountered over the past decade.
Clinical Case 1: A 45 year-old female underwent single parathyroidectomy. On POD#1, she complained of palpitations and heart rate increased to 116 BPM. Thyroid test results noted low-normal TSH [0.56 uIU/ml, n 0.5-4.8 uIU/ml] and elevated Total T4 (TT4) and Free T4 (FT4) [13.8 mcg/dL and 2.1 ng/dL, n TT4 4.5-12.0 mcg/dL and n FT4 0.9-1.5 ng/dL]. Pre-surgery and post-recovery thyroid function was normal with TSH 1.2 and 1.2 uIU/ml, TT4 10.2 and 9.4 mcg/dL and FT4 0.9 and 0.8 ng/dL.
Clinical Case 2: A 65 year-old male underwent single parathyroidectomy without complications. Calcium levels stabilized without repletion. SBP increased to 180 mm Hg on POD#1 and he experienced palpitations, chest pressure and new-onset atrial fibrillation on POD#2. Thyroid test results noted suppressed TSH [0.42 uIU/ml] and elevated TT4 and FT4 [14.2 mcg/dL and 1.99 ng/dL]. Cardiac evaluation was unremarkable. Pre surgery and post-recovery thyroid function was normal with TSH 0.92 and 0.98 uIU/ml, TT4 9.7 and 8.8 mcg/dL and FT4 1.1 and 0.9 ng/dL.
Clinical Case 3: A 58 year-old female underwent neck exploration for presumed parathyroid mass and complained of fine tremors, hyperhidrosis and palpitations on POD#2. SBP was elevated to 174 mm Hg. Thyroid test results noted suppressed TSH [0.32 uIU/ml] and elevated TT4 and FT4 [14.2 mcg/dL and 2.2 ng/dL]. Pre-surgery and post-recovery results demonstrated normal thyroid function with TSH 2.1 and 2.4 uIU/ml, TT4 8.7 and 8.7 mcg/dL and FT4 0.75 and 0.75 ng/dL.
All three patients had no history of hyperthyroidism and were started on beta-blockers to control heart rate and moderately elevated blood pressure. Thyroid functions rapidly returned to baseline normal state without treatment.
Conclusion: Clinical assessment is critical in decision to initiate treatment for apparent hyperthyroidism based on abnormal post-operative thyroid tests. When in doubt, testing of blood samples drawn within a week prior to surgery is advised. Hyperthyroxinemia may be encountered in a small percentage of cases following neck exploration for non-thyroid related reasons. Appreciation of transient hyperthyroxinemia would avoid unnecessary treatment risk of severe toxicities and committing patients to costly surveillance testing.

Nothing to Disclose: ER, NS, CM, SG, RK

Pub # P2-704

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Hypothyroidism & Thyrotoxicosis (1:30 PM-3:30 PM)

Title Age-Dependent Reference Values and Thyroid Disorders in the General Population

Author String EI Klaver, R Stienstra, IP Kema, AC Muller Kobold, MM van der Klauw, BHR Wolffenbuttel
University Medical Center Groningen and University of Groningen, Groningen, Netherlands; University Medical Center Groningen and University of Groningen, Groningen, Netherlands; University Medical Center Groningen and University of Groningen, Groningen, Netherlands

Body
Introduction: Thyroid disorders frequently occur during ageing. It is estimated that 10% of people over the age of 65 have primary hypothyroidism. We established the reference values of thyroid hormone levels and prevalence of thyroid disorders in subjects participating in the large LifeLines prospective cohort study in the general population of the Netherlands.
Methods: Participants were invited through their GP's. All 18485 participants aged 18 and above who visited the research locations between December 2009 and January 2011, were evaluated. Levels of TSH, free T4 and free T3 were measured on a Roche Modular system. Subjects using thyroxin (n = 464) and/ or antithyroid (n = 19) medication were excluded. In general our population is regarded as iodine-sufficient.
Results: Mean age of 18002 participants was 45 (range 18 - 90) years, median values (interquartile range) of TSH, FT4 and FT3 were 2.12 (1.52 - 2.95) mU/l, 15.6 (14.3 - 17.0) and 5.2 (4.8 - 5.7) pmol/l, respectively. Values of TSH were significantly lower, and of FT4 and FT3 higher ($p < 0.001$) in men. There was a significant and almost linear decline of FT3, but not of FT4, with age: in the age-group 20-25 yrs FT3 was 5.7 ± 0.7 pmol/l, in the age group over 80 yrs it was 4.85 ± 0.5 pmol/l. TSH increased from 1.98 (1.45 - 2.66) in 40-yr olds, to 2.40 (1.66 - 3.54) in those aged 80. A total of 1885 subjects (10.5%) had TSH-levels > 4.0 mU/l, and 140 (0.8%) > 10 mU/l, while 142 (0.8%) had TSH < 0.4 mU/l. 56% of those with TSH > 10 mU/l had FT4 or FT3 levels below the normal reference value, while their age was only slightly higher than the age of those with normal TSH (48 ± 2 vs. 45 ± 2 yrs, $p < 0.01$).
Conclusion: We have rigorously established reference values for thyroid hormones. There is a gradual decrease of FT3, but not FT4 with ageing, and an overall prevalence of undetected primary hypothyroidism (TSH > 10 mU/l) of 0.8% in the general western population.

Nothing to Disclose: EIK, RS, IPK, ACMK, MMvdK, BHRW

Pub # P2-705

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Hypothyroidism & Thyrotoxicosis (1:30 PM-3:30 PM)

Title Thyrotoxic High-Output Heart Failure in a Pregnant Woman with Anemia

Author String JJ Miranda, MJ Laufgraben, S Soler, A Poppas, G Gopalakrishnan
Alpert Medical School of Brown University, Providence, RI

Body Introduction: Thyrotoxicosis may cause high-output heart failure, especially in conditions that additionally increase cardiac workload. We report a case of high-output heart failure in a pregnant woman with anemia and Graves' disease.
Clinical Case: A 33 y/o G5P5 woman was diagnosed with Graves' disease based on an elevated thyroid stimulating immunoglobulin of 3.2 (normal <1.3) during her second trimester of pregnancy. Her initial free thyroxine (FT4) level was 6 ng/dL (0.77 - 1.26 ng/dL), and she was started on methimazole 20 mg daily. She presented to the hospital ten days later with shortness of breath and bilateral lower extremity edema. Transthoracic echocardiogram showed mild to moderate pulmonary hypertension (pulmonary artery pressure 55 mmHg), biatrial enlargement, and normal systolic function. Her FT4 was had improved to 2.18 ng/dL but her hemoglobin was noted to be only 8.5 g/dl. She was diuresed with intravenous furosemide and transfused 2 units of packed red blood cells for a diagnosis of iron deficiency anemia, resulting in significant improvements in her symptoms. Other etiologies of pulmonary hypertension such as pulmonary embolism were excluded. With continued treatment of her thyrotoxicosis and anemia, her heart failure remained controlled for the remainder of her pregnancy.
Conclusion: Thyrotoxicosis-induced heart failure is more likely to occur against a background of other burdens on cardiac workload. The pregnant state itself represents one such burden, and this patient had the additional burden of iron-deficiency anemia. Endocrinologists and obstetricians caring for pregnant women with Graves' disease should be aware of the potential for high-output heart failure, especially when other problems, such as anemia, arise.

(1) Sheffield JS et al., Am J Obstet Gynecol 2003; 190:211-7
(2) Froeschl M et al., Cardiovasc Pathol 2006; 14:24-27

Nothing to Disclose: JJM, MJL, SS, AP, GG

Pub # P2-706

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Hypothyroidism & Thyrotoxicosis (1:30 PM-3:30 PM)

Title Unusual Musculoskeletal Manifestations of Thyroid Disease in Adolescents -- Two Case Reports

Author String R Sherafat-Kazemzadeh, R Farias-Moeller
Georgetown University Medical Center, Washington, DC

Body Thyroid dysfunctions are often associated with non specific musculoskeletal symptoms of myopathy and generalized weakness. We describe two adolescents with initial presentations of severe hypothyroidism-induced rhabdomyolysis/myonecrosis and thyrotoxic periodic paralysis.

Case #1 was previously healthy 15-yr old female presented with severe muscle necrosis and rhabdomyolysis following marching band practice and found to be profoundly hypothyroid;TSH 77.2 mIU/ml. Congenital myopathies and other etiologies of myonecrosis were ruled out. During the acute phase, her muscle enzymes peaked at the highest levels reported in the literature;CK 34724 IU/L. Initial management with vigorous fluid replacement preserved normal renal function. Full musculoskeletal recovery was achieved 3-months after treatment with levothyroxine 100 mcg/d. Exercise-induced rhabdomyolysis has been described in military recruits, trained athletes and daily runners. Statin use, quail ingestion, EBV infection and hypothyroidism, though rare, are risk factors for rhabdomyolysis. It has been proposed that thyroxine deficiency leads to changes in muscle fibers from fast twitching type II to slow twitching type I fibers, deposition of glycosaminoglycans, poor contractility of actin-myosin units, low myosin ATPase activity and low ATP turnover, hypoperfusion of muscular vessels and muscle tissue hypoxia with low muscle energy stores. Hypothyroidism should be considered a potential cause of rhabdomyolysis in pediatric patients undergoing a myopathy work-up.

Case #2 was previously healthy 14-yr-old Asian male presented with sudden-onset generalized paralysis and hypokalemia due to thyrotoxic periodic paralysis (TPP); fT4 3.05 ng/dl, TSH 0.02 mIU/ml. Patient was admitted to PICU and treatment with β -blocker and methimazole resulted in full recovery. TPP results from intracellular shift of potassium into cells and has been seen increasingly in Asian males; reported incidence is 0.1-0.2% of thyrotoxic patients in non-Asian population to 2% in China and Japan. Male: female ratio is 20:1. Certain HLA subtypes have been identified in patients susceptible to TPP. Many patients with TPP have no obvious symptoms of hyperthyroidism which may lead to improper management. If hyperthyroidism is not treated, TPP will likely recur. Pediatricians and ER physicians need to consider this fatal but treatable condition in the differential diagnosis of patients presenting with sudden onset of hypokalemia and paralysis.

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- (2) Chowta M et al.,*Indian J Med Sci* 2008;62(12):496
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- (4) Kisakol G et al., *Endocr J* 2003;50(2):221
- (5) Barahona MJ et al., *Endocr J* 2002;49(6):621
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- (8) Halverson PB et al., *Ann Intern Med* 1979;91(1):57
- (9) Bosch X et al., *N Engl J Med* 2009;361(1):62
- (10) Sharma V et al., *Intern Med J* 2008;38(5):364
- (12) Gunther DF et al., *Eur J Pediatr* 2006;165: 320
- (13) Shih-Hua L, *Mayo Clin Proc* 2005;80(1): 99

Nothing to Disclose: RS-K, RF-M

Pub # P2-707

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Hypothyroidism & Thyrotoxicosis (1:30 PM-3:30 PM)

Title Thyroid Status and Health-Related Quality of Life in the LifeLines Cohort

Author String EI Klaver, R Stienstra, MM van der Klauw, IP Kema, BHR Wolffenbuttel
University Medical Center Groningen and University of Groningen, Groningen, Netherlands; University Medical Center Groningen and University of Groningen, Groningen, Netherlands; University Medical Center Groningen and University of Groningen, Groningen, Netherlands

Body
Background: Thyroid disorders are prevalent in western society, yet many subjects experience limited symptoms at diagnosis, especially in hypothyroidism.
Objective: To compare the health-related quality of life (HR-QOL) of subjects with suppressed TSH-levels (TSH < 0.5 mU/l) or elevated TSH-levels (TSH > 10 mU/l) to subjects with normal TSH-levels (TSH 0.5 - 4 mU/l).
Design: Cross-sectional study within the Dutch adult population who participated in the LifeLines-cohort from December 2009 until August 2010. We measured thyroid hormone status (serum TSH, free T4 and free T3, Roche Modular) and HR-QOL (RAND-36 item Health Survey) in 9491 Caucasian participants, 3993 men and 5498 women (median age 45, range 18 - 88 years), without current or former use of thyroid medication.
Results: Suppressed TSH-levels (< 0.5 mU/l) were found in 114 participants (1.2%), while 70 participants (0.7%) had TSH > 10 mU/l. No relationship between increasing TSH-levels or reduced HR-QOL and ageing could be found. Men had a higher HR-QOL than women ($p < 0.001$, Mann-Whitney) except for the domain 'general health' ($p = 0.692$). None of the domains of the RAND-36 was significantly reduced in men with suppressed or elevated TSH-levels ($p \gg 0.05$, Kruskal-Wallis) compared to euthyroid men. Only the domains 'physical functioning' and 'general health' were significantly reduced in women with suppressed TSH-levels versus euthyroid women ($p = 0.013$ and $p = 0.036$, resp., Mann-Whitney). Women with TSH > 10 mU/l had similar HR-QOL compared to euthyroid women ($p \gg 0.05$). In both men and women no significant differences could be observed between HR-QOL and subjects with decreased, normal or elevated FT4-levels ($p \gg 0.05$, Kruskal-Wallis).
Conclusion: HR-QOL of subjects with suppressed TSH-levels or elevated TSH-levels is not significantly reduced compared to subjects with normal TSH-levels, except in women with suppressed TSH-levels in the domains 'physical functioning' and 'general health'.

Nothing to Disclose: EIK, RS, MMvdK, IPK, BHRW

Pub # P2-708

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Hypothyroidism & Thyrotoxicosis (1:30 PM-3:30 PM)

Title Maintenance of Euthyroid State in Treated Hypo- or Hyperthyroid Patients Is Not Related to Carotid IMT

Author String H-J Kim, Y-J Cho, E-G Hong, J-Y Oh, SW Kim, S-Y Park
Eulji General Hospital, Eulji University School of Medicine, Seoul, Republic of Korea; Hallym University College of Medicine, Seoul, Republic of Korea; Ewha University School of Medicine, Seoul, Republic of Korea; Sungkyunkwan University College of Medicine, Seoul, Republic of Korea; Youngnam University School of Medicine, Daegu, Republic of Korea

Body It is known that some patients with mild hypothyroidism reveal the beneficial effect by levothyroxine replacement on early atherosclerotic changes. On the contrary, the others have higher carotid IMT than patients without medication. Furthermore, a linear relationship between hyperthyroidism and increased IMT caused by increased carotid artery stiffness had been reported.
The aim of this study was to investigate the status of carotid IMT in the patients with thyroid dysfunction who currently maintain euthyroid states under taking thyroxine or antithyroid drug.
One-hundred seventy three subjects(hypothyroidism n=62; hyperthyroidism n=72; control n=39) were enrolled, who had no history of diabetes, hypertension, hyperlipidemia, and any cardiovascular diseases. We divided total subjects into 5 sub-groups by the durations of medications (H1:hypothyroidism with thyroxine <5yrs, n=39; H2:hypothyroidism with thyroxine [ge]5yrs, n=33; G1:hyperthyroidism with antithyroid drug <5yrs, n=33; G2:hyperthyroidism with antithyroid drug [ge]5yrs, n=29; C:control, n=39). We assessed carotic lesions with a high-resolution ultrasound(Z.ONE Ultra,USA) using a 12-MHz linear transducer. B-mode scan were recorded on the both distal CCA and the mean IMT were calculated by averaging the 3 measurement points in each.
The mean age of subjects was 41.6±11.6yrs and the proportion of female was 81.5%. The serum free T4 and TSH were 16.0±3.7pmol/L and 2.27±3.50mU/L, and those were not significantly different among study groups. Also, there were no significant differences in the age, sex, smoking status, the fasting glucose level and lipid profiles. The mean durations of medications were H1:27±20; G1:28±16; H2:115±50; G2:101±39months, respectively. Even though there was no significant correlation, the trends of mild increase in both carotid IMT were detected in H2 and G2 compared to the other three groups, respectively {[Rt.CCA] H1:0.57±0.19; H2:0.62±0.16; G1:0.56±0.16; G2:0.60±0.18; C:0.57±0.17mm, P=ns; [Lt. CCA] H1:0.58±0.20 H2:0.65±0.20; G1:0.57±0.17; G2:0.62±0.21; C:0.57±0.14mm, P=ns}.
In conclusion, in this study the patients with thyroid dysfunction who currently maintain euthyroid states under the medications were not related with increased nor decreased carotid IMT. However, further investigations are required to address the question of whether proper treatment can improve the atherosclerosis of such individuals because elderly patients over the age of 65 were not included in this study.

Nothing to Disclose: H-JK, Y-JC, E-GH, J-YO, SWK, S-YP

Pub # P2-709

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Hypothyroidism & Thyrotoxicosis (1:30 PM-3:30 PM)

Title Hypercalcemia Associated with Hyperthyroidism

Author String CDP Blankenburg, EDS Portes, RA Guerra, ER Barbosa, LB de Sa, VC Machado, AR Andrade, DL Belino
Hospital do Servidor Publico Estadual/SP, São Paulo, Brazil

Body The association of thyrotoxicosis with hypercalcemia, bone desmineralization, osteoporosis and even pathological fractures has been described. The incidence of these findings in clinical practice, generally mild and asymptomatic, ranges from 11 to 52%. We report the case of a black woman, 49 years old, complaining o a loss of 18 kg in 3 months, with tremor of extremities, hiperdefecation, body aches, palpitations and irritability. On examination she was afebrile, very thin, with diffuse goiter and exophthalmos. Tests showed TSH 0.02 mU/L (RV: 0,3-4,0 mUI/L), FT4 8.5 ng/dl (RV:0,8-2,0 ng/dl), Ca 11.9 mg/dl (RV: 8,4-10,2 mg/dl), PTH 10.7 pg/ml (RV: 12-72 pg/ml), alkaline phosphatase 439 mg/dl (RV: 50-250 U/L), bone damage ("salt and pepper" in skull, bilateral cortical irregularities with subperiosteal reabsorption in the tibia), and bone density of lumbar spine T-score of -3.1. She was extensively investigated and discarded malignancy. Graves' disease was diagnosed and hypercalcemia was associated with hyperthyroidism. After conservative treatment with antithyroid drugs and iodine 131 ablation, serum calcium and PTH returned to normal with improvement of bone pain. In cases of thyrotoxicosis, the increase in bone reabsorption greater than that of bone formation can cause hypercalcemia without parathyroid disease, with expected suppression of serum PTH. We emphasize the importance of active search for bone metabolic changes in patients with thyrotoxicosis.

Nothing to Disclose: CdPB, EdSP, RAG, ERB, LBdS, VCM, ARA, DLB

Pub # P2-710

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Hypothyroidism & Thyrotoxicosis (1:30 PM-3:30 PM)

Title Subclinical Thyrotoxicosis Associated with Lithium Therapy

Author String M Goyal, DS Rosenthal, KH Hupart
NuHealth/Nassau University Medical Center, East Meadow, NY

Body A 30 year old male was admitted with bipolar disorder. TSH at the time of admission was normal (1.32 [mu]IU/ml). Lithium therapy (300 mg AM / 600 mg PM daily) was started and TSH was noted to be suppressed (0.03 [mu]IU/ml) four weeks later, but T4, free T4 and T3 levels remained normal at 6.47 mcg/dL, 1.27 ng/dL, and 128 ng/dL, respectively. Anti-Tg antibodies were undetectable and anti-TPO antibodies were 14 IU/ml (normal range < 35). The patient had neither a personal nor family history of thyroid disease nor any prior use of Lithium. Physical examination was consistent with euthyroidism; there were no extrathyroidal manifestations of Graves' disease. Thyromegaly was absent. A radioactive iodine uptake was <2% simultaneous with a serum TSH of 0.03 [mu]IU/ml; color Doppler sonography detected no hypervascularity. Silent Thyroiditis was diagnosed as a cause of the patient's subclinical thyrotoxicosis; this was concomitant with Lithium use and the question of a causal relationship was considered.

Lithium carbonate has been used for the treatment of bipolar disorder for many years. Although its use is causally associated with hypothyroidism and goiter formation, thyrotoxicosis has been reported to occur with its use. Graves' disease, Toxic Nodular Disease and Silent Thyroiditis have all been reported to occur in patients concurrently taking lithium carbonate therapy. A causal relationship has been suggested in some of these reports. Proposed mechanisms of action have included an exacerbation of anti-thyroid autoimmunity, an [ldquo]escape[rdquo] of thyroid hormone from an expanded intrathyroidal hormone pool consequent to Lithium induced inhibition of proteolytic hormone release, and a direct cytotoxic effect upon thyroid follicula cells. Most of the case reports which focus attention on the presence of Thyrotoxicosis during Lithium treatment have occurred in patients who have received Lithium for several months to years, and most have displayed thyroid autoimmunity. It is interesting to note that our patient had received Lithium for only four weeks at the time TSH was tested and found to be suppressed. In addition, the absence of detectable anti-Tg Ab and the presence of a titer of anti-TPO Ab not indicative of clinical autoimmune thyroid disease distinguishes this patient from other reports of this phenomenon.

The syndrome of thyrotoxicosis associated with lithium carbonate therapy merits wider recognition; potential mechanisms of this phenomenon will be discussed.

Nothing to Disclose: MG, DSR, KHH

Pub # P2-711

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Hypothyroidism & Thyrotoxicosis (1:30 PM-3:30 PM)

Title Natural History and Co-Morbidities in Subjects with Subclinical Hyperthyroidism: A Retrospective Study in Singapore

Author String SAKK Abdul Shakoar, SY Kua, ME Ching, R Hawkins, Y Kon, R Dalan
TanTock Seng Hospital, Singapore; TanTock Seng Hospital, Singapore

Body

Background:
Subclinical hyperthyroidism (SH, characterized by low serum thyrotropin (TSH) with normal serum thyroid hormones) is a common disorder with increased prevalence in older subjects¹. SH has been sub classified into 2 grades (Grade 1, TSH \geq 0.1mU/l and Grade 2, TSH < 0.1mU/l). There is no consensus regarding ideal management of SH. However, there is evidence for increased morbidities in SH, such as atrial fibrillation and osteoporosis².

Aim and method:
To evaluate the natural history of SH in multi-ethnic Singapore cohort and to assess the morbidities associated with SH. We identified SH subjects after excluding patients with known thyroid disease from patients who had thyroid function assessed from October 2005 to January 2010.

Results:
113 SH subjects (Male; 24, female; 89, mean age (\pm SD) at diagnosis; 67.2 ± 17.7 years, < 65 yrs; 42, and > 65 yrs; 71) were identified from the initial list of 6660 patients. 60 (53%) patients had grade 1 SH, whereas 53 (47%) had grade 2 SH at diagnosis. The mean TSH, free thyroxine (FT4), free triiodothyronine at diagnosis was 0.13 mU/l, 14.6 pM and 4.9 pM respectively. 52 (46%) had multinodular goiter, 15 (13%) had Graves' disease 7 (6%) had toxic nodule and 39 (35%) could not be classified.
Only 6 patients (5.3 %) progressed to overt thyrotoxicosis, while 15 remitted to euthyroid state on follow up (6 to 36 months). In patients with grade 1 SH at diagnosis (60), 37 remained in grade 1 SH, 8 became euthyroid, 13 progressed to grade 2 SH, and 2 progressed to overt thyrotoxicosis, while in grade 2 SH (53), 32 remained at grade 2 SH, 10 became grade 1 SH, 7 remitted to euthyroid state, and 4 progressed to overt thyrotoxicosis. However, no single predictive factor could be identified for progression or remission of SH. The prevalence of morbidities in SH subjects include; ischemic heart disease (16.8%), heart failure (8.9%), tachyarrhythmias (13.3%), any cardiovascular disease (28%), cerebrovascular disease (28%), osteoporosis (28%), and any fracture (15.9%). Subjects with FT4 >15 pmol/l predicted increased prevalence of any cardiovascular disease in logistic regression analysis, but became insignificant after adjusting for age.

Conclusion:
Most of SH cases in our cohort remain in subclinical state with very few progressing to overt thyrotoxicosis. Significant proportion of SH subjects have vascular disease, but this association needs to be confirmed in prospective controlled studies.

1. Hollowell, J. G., Staehling, N. W., Flanders, W. D., Hannon, W. H., Gunter, E. W., Spencer, C. A. & Braverman, L. E. 2002. Serum TSH, T(4), and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). *J Clin Endocrinol Metab*, 87, 489-99.

2. Biondi, B. & Coer, D. S. 2008. The clinical significance of subclinical thyroid dysfunction. *Endocr Rev*, 29, 76-131.

Nothing to Disclose: SAKKAS, SYK, MEC, RH, YCK, RD

Pub # P2-712

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Hypothyroidism & Thyrotoxicosis (1:30 PM-3:30 PM)

Title A Case of Thyroid Storm Covered by Diabetic Ketoacidosis

Author String N Hiroi, E Osada, M Sue, A Yoshihara, Y Ando, R Shigemitsu, R Oka, G Yoshino
Toho University, Tokyo, Japan

Body

Thyroid storm is a condition in which multiple organ dysfunction results from failure of the compensatory mechanisms of the body owing to excessive thyroid hormone activity induced by some factors in patients with thyrotoxicosis. While diabetic ketoacidosis (DKA) is an important trigger for thyroid storm, simultaneous development of DKA and thyroid storm is rare.

A 59-year-old woman who presented with a 4 months history of malaise and loss of appetite had developed nausea, vomiting and diarrhea 2 days ago. As DKA was suspected based on hyperglycemia and a positive for acetone in urine, the patient was immediately transferred to our hospital. There was no history of either diabetes mellitus or thyroid disease. Physical examination showed mild disturbance of consciousness, fever, and tachycardia. There were no other signs of thyrotoxicosis. Laboratory studies revealed a random blood glucose level of 336 mg/dL, a glycosylated hemoglobin level of 10.3%, urine acetone (3+), and metabolic acidosis. Since DKA and tachycardia associated with DKA was diagnosed, we initiated the patient on treatment with intravenous administration of rapid-acting insulin, adequate fluid replacement and continuous subcutaneous insulin infusion. Although the hyperglycemia and acidosis were immediately relieved, the disturbance of consciousness and tachycardia remained persistent. Levels of FT3 and FT4 were extremely high and TSH was below the detectable limit. TRAb was positive. The thyroid storm score of Burch & Wartofsky was 75/140, and the thyroid storm diagnostic criteria of the Japan Thyroid Association [1] were satisfied. Oral administration of potassium iodide, thiamazole and propranolol resulted in immediate relief of the tachycardia.

We encountered a case of thyroid storm associated with DKA and Graves' disease. All of these diseases can be fatal, therefore, early detection and treatment are of utmost importance. The thyroid storm diagnostic criteria prepared in 2008 by the Japan Thyroid Association are very simple as compared to the Burch & Wartofsky scoring system for thyroid storm. The Japanese criteria may be useful in the diagnosis of this condition since they enable clinicians to identify a broad range of cases with thyroid storm. We think that patients with either DKA or thyroid storm should be treated bearing in mind the possible occurrence of the two conditions in combination; we therefore consider this case as worthy of reporting.

1. The Guideline Committee for Thyroid Storm of Japan Thyroid Association and Japan Endocrine Society (2008) Diagnostic criteria for thyroid storm (1st edition), <http://thyroid.umin.ac.jp/rinsyo/crise1.pdf>. (In Japanese)

Nothing to Disclose: NH, EO, MS, AY, YA, RS, RO, GY

Pub # P2-713

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Hypothyroidism & Thyrotoxicosis (1:30 PM-3:30 PM)

Title Incidence of Thyroid Disease in Hemachromatosis Patients in the West of Ireland

Author String R Casey, BM Lynch, J Lee, V Byrnes
Galway University Hospital, Galway, Ireland

Body **Background:** The incidence of liver disease and diabetes mellitus in haemochromatosis patients is well documented. Few studies have examined the effect of iron overload on the thyroid gland. **Purpose:** To assess the incidence of thyroid disease in Haemochromatosis patients in the west of Ireland and the cost effectiveness of routine thyroid function tests in this cohort. **Methods:** A retrospective review of a haemochromatosis database from a single centre over 6 years was examined. Patients attended prior to venesection. Baseline characteristics collected included age, gender, genotype, diabetes and medication history. Serum TSH and free T4 was measured. We defined hypothyroidism as having TSH >4.2mIU/L and free T4 < 10.5pmol/L, and hyperthyroidism as having TSH < 0.27mIU/l and free T4 > 22.0 pmol/L. Values are expressed as percentage. **Results:** 568 patients with haemochromatosis were included in the database. 60.9% were male. 58.1% were HFE C282Y homozygous, 9.7% were HFE H63D homozygous and 32.2% were compound heterozygote for C282Y and H63D. The incidence of Diabetes was 4.6%. 5.6% had hypothyroidism and 0.5% had hyperthyroidism. <1% had both diabetes and thyroid disease. Ferritin at diagnosis was 184.4 units and 216.8 in the groups with and without thyroid disease respectively. 11.5% of females and 4.0% of males had thyroid disease at the time of diagnosis of haemochromatosis. **Conclusion:** The incidence of thyroid disease at the time of diagnosis in patients with haemochromatosis is very low in this Irish cohort. Our figures are in keeping with published literature. ¹ Therefore, routine screening of thyroid function at the time of diagnosis of haemochromatosis is not cost effective.

Ruth Casey, Bernadette M Lynch, Valerie Byrnes, John M Lee.
Department of Gastroenterology, Galway University Hospital, Galway, Ireland.
1. Barton et al., Thyroid (2008) 18(8):831-8

Nothing to Disclose: RC, BML, JL, VB

Pub # P2-714

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Pediatric Endocrinology: Turner Syndrome, Growth, Pituitary, Congenital Adrenal Hyperplasia & Adrenal (1:30 PM-3:30 PM)

Title Contributions of Psychopedagogical Intervention Based upon Piagetian Theory to Cognitive Development of a Turner Syndrome Girl

Author String FDCF Ricardi, LL Zaia, I Pellegrino-Rosa, JT Rosa, ITN Verreschi
Universidade Federal de São Paulo, Escola Paulista de Medicina, São Paulo, Brazil; Faculdade de Educa [ccedil]ão da Universidade Estadual de Campinas, Campinas, Brazil; Centro Universitário Funda[ccedil]ão Santo André, Santo André, Brazil; Instituto de Psicologia da Universidade de São Paulo, São Paulo, Brazil

Body

Introduction
Specific profile of reduction in visual-space perception among subjects with Turner syndrome (TS) has been described. TS girls seem to have cognitive compensation of this visual deficit throughout their growing-up. With the hypothesis of a gradual increasing to this mechanism, a psychopedagogical intervention was planned in order to optimize the heteromodal connections of brain areas, associated to the development of neuropsychological compensation.

Materials and Methods
Two age-paired 45,X girls were evaluated at two moments for the Bender test (BT) and specific Piaget's tasks (the latter apparently have been applied to TS carriers only once before; Ricardi et al., 2010). In between, the experimental intervention (environmental solicitation) was applied for one year, according to Piaget's theory for thinking development. The performance on the Narrative Construction activity was analyzed for both subjects, experimental and control.

Results and Discussion
There was a difference on BT performance in favor of the experimental subject (ES), who presented more ability to planning and organizing her graphic expression. ES also showed improvement on Piagetian tasks (e.g. conservation and measure of volumes) and on Narrative Construction performance. The psychopedagogical intervention may have contributed to the performance increment of the TS girl. The visuo-motor perception analysis enhanced the understanding of TS, mainly the influence of the perceptio and motor organization of the paper surface, and the form to show the essential of the drawings on the paper.

Conclusion
According to Bender (1938), the gestalt visual-motor functions basically may be associated with language aptitude, time and space organization, and to manual motor abilities. One concluded there was an improvement on the quality of gestalt functions, observed in the paper organization and drawings planning, and that psychopedagogical activities had a strong influence on the Bender test organization and planning, so contributing to ameliorate cognitive performance of a girl with Turner syndrome.

Bender L. A visual motor Gestalt test and its clinicial use. Am. Orthopsych. Res. Monograph 1938; 103.
Ricardi FCF, Zaia LL, Pellegrino-Rosa I, Rosa JT, et al. Psychogenetics of Turner syndrome: an investigation of 28 subjects and respective controls using the Bender test and Piagetian scales. Genet Mol Res 2010; 9: 1701-1725.

Sources of Research Support: FAPESP; CAPES.

Nothing to Disclose: FdCFR, LLZ, IP-R, JTR, ITNV

Pub # P2-715

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Pediatric Endocrinology: Turner Syndrome, Growth, Pituitary, Congenital Adrenal Hyperplasia & Adrenal (1:30 PM-3:30 PM)

Title Cardiovascular Diseases in Turner Syndrome (A Single-Center Study)

Author String E Klaskova, J Zapletalova, J Wiedermann, J Kozak
Palacky University and University Hospital, Olomouc, Czech Republic; Palacky University and University Hospital, Olomouc, Czech Republic

Body **The aim of study:** To evaluate the prevalence of cardiovascular abnormalities in Turner syndrome (TS).
Background: TS is the most common chromosomal abnormality, affecting 1/2000 live female birth. Congenital heart disease (CHD) affects approximately 50% of TS individuals and it is the major cause of premature morbidity and mortality in TS adults. Recent data report an association of TS with a generalised vasculopathy and aortic abnormalities such as aortic dilatation (AoDil) and dissection. The higher risk of CHD is associated with severe dysmorphic features. Bicuspid aortic valve (BAV), aortic coarctation (CoA), aortic root dilatation (ARD) and arterial hypertension (AH) are the risk factors for aortic dissection. Risk of the aortic dissection is increased in young women particularly during pregnancy. Accurate data on prevalence of aortic valve abnormalities, AH and AoDil are not available yet.
Patients and methods: Forty four individuals with TS (18 with karyotype 45,X) age 7.4 (0.1 to 19.0) years underwent complete cardiologic examination including ECG, echocardiography, 24 hours monitoring of arterial blood pressure and 38 of them also magnetic resonance imaging (MRI). The median of age at first cardiologic evaluation was 12.3 (0.1-19.0) years.
Results: BAV or dysplastic aortic valve had 29% of subjects, CoA had 2% of patients, aortic stenosis 2% of subjects, AoDil in 18% and AH in 11% of subjects. Ascending/descending aortic diameter ratio > 95th percentile was found in 21 % individuals. Any type of cardiac abnormalities was found in 61% of all TS patients (in 78% of 45,X).
Conclusion: One third of our patients had at least one risk factor for aortic dissection, but 44,5% in the group of 45,X individuals. Our data confirm the high risk for cardiovascular diseases in TS. The patients should be carefully examined just after diagnosis of TS to detect the cardiovascular abnormalities and to identify many asymptomatic individuals with risk factors. Regularly follow-up by experienced cardiologist is necessary for early detection of cardiac abnormalities to prevent cardiovascular morbidity and mortality. MRI is recommended in all TS girls at an age when it may be performed without sedation.

Sources of Research Support: Research Project SGS UP LF_2011_018.

Nothing to Disclose: EK, JZ, JW, JK

Pub # P2-716

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Pediatric Endocrinology: Turner Syndrome, Growth, Pituitary, Congenital Adrenal Hyperplasia & Adrenal (1:30 PM-3:30 PM)

Title Slipped Capital Femoral Epiphysis -- An Unusual Side Effect of GnRH Agonist Therapy?

Author String EA Cummings, TE Pinto, AS Mokashi
Dalhousie University, Halifax, Canada

Body **Background:** Slipped capital femoral epiphysis (SCFE) is known to be associated with endocrine disorders, most commonly growth hormone (GH) deficiency and hypothyroidism. An association with Gonadotropin-releasing hormone agonist (GnRHa) treatment is less clear. Of 6 previously reported cases, SCFE occurred in 4 after withdrawal of GnRHa, leading some authors to conclude that the mechanism involved relates to increased growth plate activity in a weakened epiphysis after withdrawal of GnRHa. We report 2 cases of SCFE in girls with central precocious puberty (CPP) while on effective treatment with GnRHa.

Clinical Cases: **Case 1** is an otherwise well child who presented with idiopathic CPP at age 22 months and began Leuprolide Acetate Depot (LAD) 7.5 mg q 3 weeks at age 2 years. After 6.75 years of continuous treatment, she presented with right SCFE at age 8.75 years. Bone age was advanced at 11 years, at 8.0 years of age, prior to presentation with SCFE. Her gonadal axis was well suppressed with estradiol of 33.8 pmol/l (N: <100) and peak LH <0.2 IU/L on a GnRH stimulation test. Thyroid function and IGF1 were normal. BMI Z-score was overweight at +1.75. **Case 2** is a child with short stature who underwent bone marrow transplant for acute lymphoblastic leukemia at age 9 months. CPP was diagnosed and LAD 7.5 mg q 3 weeks was started at age 7.3 years. Due to extreme distress regarding injections, this was changed to LAD 22.5 mg q 3 months after the first few doses. She presented with left SCFE at age 10.5 years after 3.3 years on GnRHa, at which point her bone age was 12 years and puberty was well controlled. Repeated GH stimulation and thyroid function testing was normal. BMI Z-score was normal at +0.71.

Clinical Lesson: These cases are important for 2 reasons. Firstly, they add to a small but accumulating body of evidence suggesting an association between GnRHa therapy and SCFE. Secondly, with the addition of our 2 patients to the literature, 4 of 8 cases have presented during GnRHa therapy. It, therefore, can no longer be concluded that the major mechanism for SCFE in this clinical situation relates to increased growth velocity after GnRHa therapy in a weakened epiphysis. Rather the lack of normal sex steroid levels in the epiphysis at a critical bone age may be of greater importance. Endocrinologists should be aware of the potential association of GnRHa therapy with SCFE and counsel families regarding signs and symptoms during, and after, GnRHa therapy.

Nothing to Disclose: EAC, TEP, ASM

Pub # P2-717

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Pediatric Endocrinology: Turner Syndrome, Growth, Pituitary, Congenital Adrenal Hyperplasia & Adrenal (1:30 PM-3:30 PM)

Title Significant Gain in Final Height in Brazilian Turner Syndrome (TS) Patients Treated with rhGH in Comparison with Untreated Patients

Author String RC Scalco, AF Braz, G Guerra-Junior, SRR Antonini, AC Malaquias, SHV Lemos-Marini, AD Baldin, E Ramos, CE Martinelli-Junior, IJP Arnholt, BB Mendonca, AAL Jorge
Hospital das Clinicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil; Faculdade de Medicina da Universidade de Campinas, Campinas, Brazil; Faculdade de Medicina de Ribeirao Preto, Universidade de São Paulo, Ribeirao Preto, Brazil

Body
Background: Short stature is the most prevalent feature of TS and recombinant human growth hormone (rhGH) is an approved therapy for this condition in several countries. However there is a paucity of studies that measure the magnitude of effectiveness of rhGH therapy in TS in special regarding the final height.
Objective: To assess the efficacy of rhGH therapy to improve final height of TS patients.
Patients and Methods: Final heights of 182 TS patients (73 rhGH-treated and 109 untreated) and of 133 healthy women (control group) were assessed. Information about karyotype, parents' height and puberty development was recovered in TS patients. Pre-treatment clinical data were retrospectively obtained to identify variables that could predict the growth response in rhGH treated group. Final height was determined when the growth rate was <1 cm/y observed during a minimum 12 months of follow-up.
Results: TS treated group started rhGH (mean dose of 48 [micro]g/kg.d) at a chronological age (CA) of 11 ± 3 yr. bone age 9 ± 3 yr, height SDS (British 1966 standards) -3.0 ± 0.9 and were treated for 5 ± 2 yr. Estrogen replacement was started at CA 14.4 ± 2.0 yr, after 3.6 ± 2.2 yr of rhGH therapy. The age at start of puberty and the proportion of patients with spontaneous puberty (16%) was similar in treated and non-treated TS patients. At adult age, final height of rhGH-treated TS group was 151 ± 5 cm and in non-treated group was 143 ± 7 cm. TS patients were significantly shorter than control group (165 ± 5 cm, $p < 0.001$). When final height was corrected for target height (TH), the mean differences in height between control group and rhGH-treated and non-treated TS patients were -9.5 and -18.1 cm respectively. The effect of rhGH therapy on final height was a gain of 8.3 cm (95%CI of 4 to 13 cm). Treated patients had a total height SDS increment of 1.1 relative to pretreatment (95%CI 0.8 to 1.4, $p < 0.001$). In a forward stepwise regression, the independent variables CA at start of puberty ($p < 0.001$), pre-therapy height SDS ($p < 0.001$), BMI SDS ($p = 0.022$), height SDS gain during the first year of rhGH treatment ($p = 0.006$) and TH ($p = 0.015$) explained 66% of the variability of final height.
Conclusion: Despite of a relatively late onset of rhGH therapy in our TS cohort, treated patients obtained an important height gain attributed to rhGH therapy. Estrogen replacement delayed associated with higher rhGH doses, might explain the good results observed in the present study.

Nothing to Disclose: RCS, AFB, GG-J, SRRA, ACM, SHVL-M, ADB, ER, CEM-J, IJPA, BBM, AALJ

Pub # P2-718

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Pediatric Endocrinology: Turner Syndrome, Growth, Pituitary, Congenital Adrenal Hyperplasia & Adrenal (1:30 PM-3:30 PM)

Title Influence of Parental Origin of the X Chromosome on Clinical Features, Associated Complications and the Two-Year Response to Growth Hormone (rhGH) of Patients with Turner Syndrome (TS)

Author String F Alvarez, R Lanes, JM Quintero, M Miras, H Fideleff, V Mericq, H Marcano, W Zabala, M Soto, T Pardo, I Borjas, J Villalobos, P Gunczler, N Unanue, N Tkalenko, A Boyanofsky, L Silvano, L Franchioni de Munoz, M Llano, G Fideleff, M Azaretzky, M Suarez
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Body **BACKGROUND:** TS is defined as the combination of short stature, gonadal dysgenesis, typical visible dysmorphic stigmata, and urinary, cardiovascular, and skeletal abnormalities associated with a missing or structurally abnormal second sex chromosome. The clinical features of TS are variable and previous studies have suggested that the parental origin of the X-chromosome has effects on phenotypic features of TS in subjects 45,X.
OBJECTIVE: We studied the influence of the parental origin of the X-chromosome on clinical features, associated complications and the two-year response to rhGH in patients with TS and a 45,X karyotype.
DESIGN, PATIENTS AND METHODS:
This work was part of a Latin-American multicenter prospective study. TS patients with a 45,X karyotype (n=40) and a mean age of $8,66 \pm 4,08$ yrs and both their parents were enrolled. We determined the parental origin of the normal X-chromosome and correlated this origin with clinical features including anthropometric data, congenital malformations, biochemical profiles and growth velocity at the beginning and after two years of rhGH treatment. DNA polymorphisms using X-STR decaplex were analyzed to determine the parental origin.
RESULTS
X-STRs analyses determined that 70% (28/40) of the patients retained a normal maternal X chromosome. Three subjects had an occult mosaicism. We were unable to demonstrate the parental origin effect on clinical features including anthropometric data (birth weight-height, body mass index, bone age), congenital malformations (renal, cardiac, auditive or ocular systems), and biochemical or thyroid profiles. Clinical data at the start or after the first two years of rhGH therapy were indistinguishable among patients with different retained X chromosomes (maternal or paternal X chromosome). A positive correlation between the patient's height and maternal height (expressed in SDS) was demonstrated in individuals with a maternal retained X chromosome ($r= 0.527$, $P [le]0.001$). However, a correlation between the patient's height and paternal height in individuals with paternal retained X chromosome was not noted.
CONCLUSIONS
We found no significant effect of the parental origin of the X chromosome on clinical features in patients with TS. It is, however, possible that stature is more influenced by maternal than paternal height.

Nothing to Disclose: FA, RL, JMQ, MM, HF, VM, HM, WZ, MS, TP, LB, JV, PG, NU, NT , AB, LS, LFdM, ML, GF, MA, MS

Pub # P2-719

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Pediatric Endocrinology: Turner Syndrome, Growth, Pituitary, Congenital Adrenal Hyperplasia & Adrenal (1:30 PM-3:30 PM)

Title Transition from Pediatric to Adult Health Care in Young Women with Turner Syndrome

Author String KC Garvey, JA Finkelstein, JI Wolfsdorf, ET Rhodes
Children's Hospital Boston, Boston, MA; Harvard Medical School, Boston, MA

Body Turner syndrome (TS) requires lifelong follow-up with attention to multiple organ systems. European studies have shown suboptimal follow-up in young adults with TS, but there are no published data on health care transitions for TS in the United States. We developed a survey to evaluate characteristics of the transition from pediatric to adult care in women with TS (including reasons for transition, transition preparation and education, patient satisfaction and perceived barriers) as well as post-transition medical care, support structure, and barriers to self-care. We mailed the survey to the last known address of the 46 women with TS seen in the last 10 years in the Divisions of Endocrinology or Adolescent Medicine at Children's Hospital Boston who were over 15 years old at the time of their last visit and who had not been seen in the past 18 months as of May 2010. We sent surveys in 3 waves between October and December 2010. As of January 2011, 10 surveys (22%) were undeliverable and the response rate was 31% (11/36). Respondents were 25.4 ± 1.9 years old and reported transition at 18.0 ± 2.7 years; 45% currently lived with their parents. While all had seen an adult medical provider in the past year, only 5 (45%) received any medical care for TS in the year after transition, and in the years prior to the survey, only 4 (36%) had received all necessary TS screening test at the intervals recommended by national clinical practice guidelines. The most commonly cited reasons for initiating transition were suggestion by pediatric providers (72%) or parents (55%), and 73% of respondents reported that they were "mostly" or "completely satisfied" with their transition. Most (73%) respondents felt that their pediatric provider had discussed independent TS management, but a specific adult provider/practice was recommended to only one patient and 45% had never talked to their pediatric provider without a parent in the room. Nonetheless, the majority of respondents rated these latter two issues as important parts of transition. Parents were rated as "moderately" or "very" involved in adult TS care by 73% of patients, and this involvement matched the respondents' stated preference in all cases. We conclude that further research in TS transition should focus on patient preparation by pediatric providers, post-transition gaps in care, and the degree of parental involvement versus self-care.

Nothing to Disclose: KCG, JAF, JIW, ETR

Pub # P2-720

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Pediatric Endocrinology: Turner Syndrome, Growth, Pituitary, Congenital Adrenal Hyperplasia & Adrenal (1:30 PM-3:30 PM)

Title Size at Birth and Postnatal Growth Are Associated with IGF-I Levels between Birth and Six Months' Corrected Age in Preterm Infants

Author String M van de Lagemaat, J Rotteveel, MM van Weissenbruch, HN Lafeber
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Body

Introduction: Preterm infants are susceptible to postnatal growth restriction. Fetal and early postnatal growth are regulated by insulin-like growth factor type 1 (IGF-1). IGF-1 is positively associated with size at birth and postnatal growth in term infants. The relation between size at birth, postnatal growth, and IGF-1 levels has not been studied yet in preterm infants.

Objective: To study IGF-1 levels in relation to size at birth and postnatal growth until 6 months post term in preterm infants.

Material and methods: Anthropometry at birth, term, 3 and 6 months and IGF-1 levels at term, 3 and 6 months were measured in 139 preterm infants (51% male; boys: gestational age (GA) 30.5 ± 1.3 wk, birth weight (BW) 1374 ± 284 g (-0.5 ± 1.1 SD); girls: GA 30.1 ± 1.6 wk, BW 1306 ± 291 g (-0.2 ± 0.8 SD)). Postnatal growth was classified as preterm growth restraint (PGR; weight (W) and length (L) [ge]-2 SD at birth and W and/or L <-2 SD at term), non-PGR (W and L [ge]-2 SD at birth and term), small-for-gestational-age (SGA; W and/or L <-2 SD at birth and term), and SGA with catch-up growth (SGA-CUG; W and/or L <-2 SD at birth and W and L [ge]-2 SD at term).

Results: Weight and length at birth were associated with IGF-1 levels at term (resp. $\beta=0.59$, CI 0.29-0.91, $p=0.000$ and $\beta=0.44$, CI 0.16-0.71, $p=0.002$). IGF-1 levels were positively associated with head circumference at term and 3 months (resp. $\beta=0.15$, CI 0.07-0.24, $p=0.000$ and $\beta=0.56$, CI 0.13-0.99, $p=0.011$) and with weight and length at term, 3 and 6 months (term: weight $\beta=0.31$, CI 0.21-0.41, $p=0.000$ and length $\beta=0.29$, CI 0.18-0.39, $p=0.000$; 3 months: weight $\beta=1.37$, CI 0.83-1.92, $p=0.000$ and length $\beta=1.08$, CI 0.59-1.58, $p=0.000$; 6 months: weight $\beta=0.75$, CI 0.26-1.25, $p=0.003$ and length $\beta=0.59$, CI 0.13-1.05, $p=0.012$). These associations were not influenced by gender.

At term, PGR and SGA infants had significantly lower IGF-1 levels than SGA-CUG and non-PGR infants (5.1 ± 1.5 and 4.7 ± 0.9 versus 7.3 ± 1.6 and 7.0 ± 1.8 nmol/l resp.). At 3 months, only SGA infants had significantly lower IGF-1 levels than SGA-CUG and non-PGR infants (6.29 ± 1.77 versus 10.09 ± 4.21 and 9.37 ± 3.77 nmol/l resp.). At 6 months, IGF-1 levels were similar in PGR groups.

Conclusion: In preterm infants, IGF-1 levels predict head circumference during the first 3 months as well as length and weight during the first 6 months post term. As a result of catch-up growth, PGR classification was associated with IGF-1 levels at term and 3 months, but not at 6 months.

Nothing to Disclose: MvdL, JR, MMvW, HNL

Pub # P2-721

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Pediatric Endocrinology: Turner Syndrome, Growth, Pituitary, Congenital Adrenal Hyperplasia & Adrenal (1:30 PM-3:30 PM)

Title Confirmation of a Congenital Syndrome of Severe Hypoglycemia and Overgrowth with Undetectable Plasma Insulin

Author String K Hussain, M Minic, B Challis, J Harris, S O'Rahilly, U Ramaswami, P De Lonlay, RK Semple
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Body We have previously described a patient with severe fasting hypoglycemia, suppressed ketones and concomitantly undetectable plasma insulin, necessitating long term percutaneous feeding overnight. The severe metabolic derangement was associated with neonatal macrosomia and progressive overgrowth. We now report three further patients with recurrent symptomatic fasting hypoglycaemia in infancy, in all cases associated with suppressed ketones and low or normal fatty acids but undetectable plasma insulin. In one patient this was investigated further by comprehensive pancreatic and mesenteric venous sampling at the time of severe hypoglycemia, confirming undetectable insulin and normal IGF1 levels. Three of the four patients described also had neonatal macrosomia, and three had left sided hemihypertrophy. One patient had cystic nephropathy and a neonatal adrenal carcinoma that was curatively resected. Detailed investigation failed to reveal any known inborn error of metabolism, and no evidence of 11p15 abnormalities consistent with Beckwith Wiedemann syndrome was found. None of the three new patients had any evidence of increased IGF1 or IGF2, with moreover no evidence of abnormally processed [ldquo]big[rdquo] IGF2. Despite a metabolic profile consistent with insulin-like action and undetectable plasma insulin, the sequence of the *INS* gene, encoding the insulin receptor, was normal in all four patients. We thus describe a novel form of severe fasting hypoglycaemia with biochemical features of insulin action but undetectable plasma insulin and progressive overgrowth. Possible explanations include abnormal paracrine or endocrine activation of the insulin receptor, or constitutive activation of the insulin signalling pathway by a novel mutation.

Sources of Research Support: Wellcome Trust; UK Medical Research Council Centre for Obesity and Related Disorders; NIHR Cambridge Biomedical Research Centre.

Nothing to Disclose: KH, MM, BC, JH, SO, UR, PDL, RKS

Pub # P2-722

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Pediatric Endocrinology: Turner Syndrome, Growth, Pituitary, Congenital Adrenal Hyperplasia & Adrenal (1:30 PM-3:30 PM)

Title A New Intragenic Micro Deletion of the IGF-I Receptor Gene, in an Italian Family with Developmental and Reproductive Defects

Author String F Phan-Hug, F Zufferey, M Bellavia, D Martinet, F Niel Buttschi, S Gerasimos, PE Mullis, N Pitteloud, S Jacquemont
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Body **Background:** IGF1R mediates the actions of IGF1 and IGF2 in controlling proliferation and differentiation of multiple tissues. IGF1-Receptor (IGF1R) deficiency causes intrauterine and postnatal growth retardation in mice and men.
Methods and results: This study investigates a 3 generation consanguineous Italian family (n=9) with short stature (< 4 SDS). A comparative genome hybridization (CGH) array in the one-year old male proband and his mother revealed a heterozygous micro deletion of at least 81 kb on 15q26.3 encompassing exons 3-13 of IGF1R (MIMID 147370) resulting in IGF1R haploinsufficiency. The proband was small for gestational age (birth weight: 2060gr, - 4 SDS; birth length 42 cm, - 4 SDS), with severe microcephaly (head circumference: 30 cm, - 4 SDS) and clinobrachydactyly. Postnatal growth was reduced with significantly delayed bone maturation. Levels of IGF1 (106 mcg/l [56-350; 2.5-97.5 perc.]) and IGFBP-3 (3.08 mg/l [0.7-3.5; 2.5-97.5 perc.]) were normal. Psychomotor development at one year was normal.
The mother presented with a normal birth weight (2500 gr, -1.69 SDS) markedly short stature (137 cm, - 4.5 SDS), normal weight (42 kg, -1.5 SDS), severe microcephaly (49 cm, - 4.69 SDS), clinobrachydactyly, and a slightly reduced IQ of 75. Serum IGF1 was elevated (386 mcg/l [106-277; 2.5-97.5 perc.]) while IGFBP-3 was at the upper range of normal (6.4 mg/l [3.4-6.6; 2.5-97.5 perc.]). Because of a 5-year history of infertility and irregular menses she was diagnosed with decreased ovarian reserve at age 35 based on a day-3 serum FSH of 11 IU/L, normal prolactin and TSH and no signs of polycystic ovarian syndrome. FMR1 gene testing was normal. She underwent 3 IVF cycles with poor response in terms of gonadotropin stimulation and fecundity yet conceived a son. Notably, both her parents exhibit severe short stature: grandfather, 149cm (-4.24 SDS); grandmother 143cm (-3.61 SDS). Additionally, the grandfather also presented with microcephaly (52.5 cm, - 3.06 SDS).
Clinical lessons and conclusion: We describe a new intragenic microdeletion of the IGF1R gene in a family with severe small stature and microcephaly and a novel female reproductive phenotype. This heterozygous deletion implies an autosomal dominant mode of inheritance. However, as both maternal grandparents have small stature, a more complex genetic model in this family regarding this phenotype is possible. Further genetic investigations are underway.

Nothing to Disclose: FP-H, FZ, MB, DM, FNB, SG, PEM, NP, SJ

Pub # P2-723

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Pediatric Endocrinology: Turner Syndrome, Growth, Pituitary, Congenital Adrenal Hyperplasia & Adrenal (1:30 PM-3:30 PM)

Title Evaluation of Insulin Secretion and Sensitivity and Lipid Profile in Growth Hormone (GH)-Deficient Children before and at the End of rhGH Therapy

Author String S Bellone, F Prodam, S Savastio, A Petri, C Balossini, G Genoni, R Ricotti, M Gatto, G Aimaretti, G Bona
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Body

Introduction. A rise in serum insulin levels during GH therapy is reported. Insulin resistance is a risk factor for type 2 diabetes, atherosclerosis, dyslipidemia, and hypertension. Few data describe insulin secretion and lipid after the end of GH therapy.

Subjects and methods. Aim of our study was to evaluate changes in insulin secretion, sensitivity and lipid profile in children with isolated GHD longitudinally followed during rhGH treatment. We measured lipid profile, glucose and insulin levels at fasting and after an oral glucose tolerance test (OGTT) in 24 GHD children at 4 times: 1) before starting of GH therapy (BT); 2) during the last year of therapy (T1); 3) 6 months (T6) and 4) 12 months (T12) after stopped therapy. They were compared at T12 with 28 healthy puberty matched subjects.

Results. No subjects showed dysglycemia or hypertension. Basal glucose levels were lower at BT but similar among the last 3 times. Fasting insulin, insulin and glucose levels at each point after OGTT were progressively lower at T6 and T12 compared to T1 ($p < 0.02$). HOMA-IR index was higher, and Matsuda and QUICKI indexes were lower at T1 respect to T6 and T12 ($p < 0.02$) without returning to the BT prepubertal levels. A compensatory insulin secretion was recorded at T1 as HOMA-B and insulinogenic index both of which progressively decreased at T6 and T12 respect to T1 ($p < 0.04$) returning to BT levels. The disposition index was unchanged from BT to T12. HDL-cholesterol decreased after stopped therapy ($p < 0.04$). All the parameters were similar to controls at T12.

Conclusions. Insulin sensitivity decreased during rhGH therapy without the onset of dysglycemia and was associated with a compensatory insulin secretion after OGTT and without changes of the disposition index. Insulin and glucose secretion progressively restored after stopping treatment being similar to controls in the next 12 months.

Nothing to Disclose: SB, FP, SS, AP, CB, GG, RR, MG, GA, GB

Pub # P2-724

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Pediatric Endocrinology: Turner Syndrome, Growth, Pituitary, Congenital Adrenal Hyperplasia & Adrenal (1:30 PM-3:30 PM)

Title Functional Characterization of a Novel Homozygous Mutation in the Insulin-Like Growth Factor-1 Receptor Identified in a Child Born Small for Gestational Age

Author String JM Volkmann, J Klammt, M Schlicke, H Stobbe, S Putzker, HP Schwarz, R Pfaffle
University of Leipzig, Leipzig, Germany; University of Munich, Munich, Germany

Body

Background: The IGF1 receptor (IGF1R) plays an essential role in intrauterine and postnatal growth regulation. To date, several heterozygous mutations in the IGF1R leading to IGF1 resistance have been described. In mice, homozygous *Igf1r* knock out results in perinatal death due to respiratory failure. We now present the first homozygous mutation in a girl with intrauterine and postnatal growth retardation.

Objective: Aim of the study is to explore the impact of a novel homozygous IGF1R mutation identified in a girl born small for gestational age (SGA) by genetic, comparative and experimental approaches.

Patient: The girl was born in the 41st week of gestation with a length of 44 cm (-3.16 SDS) and a weight of 2210 g (-2.35 SDS). Two years later she had not caught up the initial growth deficit. Clinical examination revealed no additional phenotypic abnormalities. IGF1 and IGFBP3 levels were elevated [at 3 yrs: 335 ng/ml (51-303), 4.3 [micro]g/ml (0.7-3.6)].

Results: Denaturing HPLC pre-screening and direct DNA sequencing of the index patient's genomic DNA disclosed a homozygous *IGF1R* mutation resulting in an amino acid exchange at position 56 from glutamic acid to aspartic acid (p.E56D). Her parents were found to carry the mutation in a heterozygous state. The highly conserved residue is located in the extracellular ligand binding domain (L1) of the receptor. *In vitro* analyses in *Igf1r* deficient R-cells transiently transfected with wild type or mutant receptor plasmids showed that the mutation results in a decreased autophosphorylation of the receptor in response to IGF1 and a diminished protein kinase B/Akt activation compared to the wild type. Flow cytometry assays performed in transiently transfected Cos-7 cells demonstrated a decrease in the mutant receptor cell surface expression compared to the wild type.

Conclusion: The E56D mutation identified in a patient with typical features of IGF1 resistance diminishes the autophosphorylation of the IGF1R and subsequently leads to a moderate decline in the activation of the downstream signalling. The hypomorphic effect of the mutation might be due to decreased receptor expression on the cell surface. Assays to determine the IGF1 binding properties of the receptor are in progress. The conservative amino acid substitution is suggested to provoke the growth retarded phenotype. However, it does not result in a complete loss of receptor function and, even in the homozygous state, might therefore be compatible with life.

Sources of Research Support: Grant of the Deutsche Forschungsgemeinschaft (DFG PF 225/3-2) to R.P. and J.K.

Nothing to Disclose: JMV, JK, MS, HS, SP, HPS, RP

Pub # P2-725

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Pediatric Endocrinology: Turner Syndrome, Growth, Pituitary, Congenital Adrenal Hyperplasia & Adrenal (1:30 PM-3:30 PM)

Title Hypopituitarism in Pediatric Survivors of Inflicted Traumatic Brain Injury

Author String BA Auble, K Makoroff, T Weis, T Colliers, SR Rose
Cincinnati Children's Hospital Medical Center, Cincinnati, OH; Cincinnati Children's Hospital Medical Center, Cincinnati, OH

Body

Background:
There is growing evidence of endocrine dysfunction in adults after traumatic brain injury (TBI). However, limited data in children suggest that the prevalence of hypopituitarism following TBI in children is 10-61%. Normal pituitary function is necessary for growth and normal onset of puberty. It is not known to what degree inflicted traumatic brain injury (iTBI) causes hypopituitarism in children.

Methods:
To date, 11 patients with history of iTBI were studied by a multidisciplinary team to evaluate pituitary function. Only patients injured before 3y of age were included. Inclusion criteria included: iTBI requiring hospitalization, [ge] 1 year since injury, and >2 to 9 y of age. Evaluation comprised: overnight hospital stay, height and weight measurements, and blood samples for cortisol peak to cosyntropin, free T4, thyroid stimulating hormone nadir to peak rise at night (TSH surge), 6h every 20 minute growth hormone (GH) pulses, IGF-I, IGFBP3, prolactin, and serum/urine osmolality.

Results:
iTBI led to all patients having subdural hematoma, while 7 of the 11 patients had CT evidence of edema or ischemia. All but one required ICU care, 10 had seizures, and 7 required intubation. Nine (81%) showed at least one endocrine abnormality including low height, elevated TSH, decreased TSH surge, low GH peak, or elevated prolactin. Seven (64%) had more than one abnormal pituitary function.

Conclusions:
In this small pilot study of children following iTBI, almost every patient revealed at least one abnormal measure of pituitary function. This suggests that patients who have suffered iTBI should be monitored by clinical evaluation, including length or height measurements, and assessment of pituitary function.

Sources of Research Support: In part by Pfizer, Inc, as an independent Investigator-Initiated Research grant, in part by General Clinical Research Center, grant #M01 RR08084 from NIH, and in part by USPHS Grant #UL1 RR026314 from the National Center for Research Resources, NIH.

Disclosures: BAA: Clinical Researcher, Pfizer, Inc. TW: Coinvestigator, Pfizer, Inc. SRR: Clinical Researcher, Pfizer, Inc. Nothing to Disclose: KM, TC

Pub # P2-726

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Pediatric Endocrinology: Turner Syndrome, Growth, Pituitary, Congenital Adrenal Hyperplasia & Adrenal (1:30 PM-3:30 PM)

Title The Frequency and Severity of Endocrine Health Conditions Observed in Pediatric Brain Tumor Survivors

Author String BC Patterson, A Mertens, K Wasilewski-Masker, LR Meacham
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Body

Background. National guidelines direct the surveillance of survivors of pediatric cancers for late effects of cancer therapy, including endocrine late effects. Surveillance is individualized and agent-specific. Endocrine surveillance is recommended after alkylating agents, methotrexate, steroid cancer therapy and radiation to endocrine organs. The purpose of this study was to describe the endocrine outcomes in a cohort of brain tumor survivors compared to other pediatric cancer survivors followed in a program utilizing these guidelines.

Methods. Pediatric and young adult brain tumor survivors were recruited prospectively from patients referred to the Brain Tumor Survivor Program (BTSP). Patients seen in the BTSP were evaluated with history, physical and testing as per the Children's Oncology Group *Long-Term Follow-Up Guidelines for Survivors of Childhood, Adolescent and Young Adult Cancers*. Data collected included demographic information, cancer treatments, and list of health conditions. Endocrine conditions were scored for severity (1, mild to 5, death) according to the Common Terminology for Adverse Events v.4.03. Brain tumor survivors were compared to other pediatric and young adult survivors of non-central nervous system childhood cancers seen in the Cancer Survivor Program. Brain tumor survivors (n=28) were matched 1:2 to other survivors (n=56) on gender and age at cancer diagnosis.

Results. 89% of brain tumor survivors had at least one endocrine condition, compared to 59% of other survivors (p=0.005, Fisher exact test). The most common endocrine conditions in brain tumor survivors were hypothyroidism (n=17) and hypogonadism (n=9). The mean number of endocrine conditions per survivor was higher in brain tumor survivors (2.9) than other survivors (0.8) (t-test, p<0.001). In a linear regression model, radiation (p=0.02) and time between diagnosis and BTSP visit (p=0.03) were associated with an increased number of endocrine conditions ($R^2=0.30$). Among brain tumor survivors, most endocrine conditions were mild (Grade 1) or moderate (Grade 2); there were 6 severe or life-threatening conditions (Grade 3 or 4) and no deaths.

Conclusions. Endocrine conditions occur commonly after pediatric brain tumors. Endocrinologists should be aware of the *COG-LTFU Guidelines*, anticipate referral of pediatric brain tumor survivors, and participate in further research to identify the best strategies to diagnose and treat the endocrinological late effects of cancer therapy.

Nothing to Disclose: BCP, AM, KW-M, LRM

Pub # P2-727

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Pediatric Endocrinology: Turner Syndrome, Growth, Pituitary, Congenital Adrenal Hyperplasia & Adrenal (1:30 PM-3:30 PM)

Title A Female 46,XY Patient with 17 α -Hydroxylase/17,20-Lyase (P450c17) Deficiency with Pubertal Development and Bilateral Seminoma

Author String LS Harima, EMF Costa, AZ Machado, FM Carvalho, S Domenice, BB Mendonca
Endocrinologia Hospital das Clinicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil; Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil

Body Background: 46,XY patients with disorders of sex development (DSD) have a high risk factor for gonadal tumors' development, however germ cell tumors have not been previously reported in 46,XY P450c17 deficient patients. Although a gonadoblastoma (1) and a mixed germ cell tumor (2) have been described in two 46,XX P450c17 deficient patients. We described a 46,XY patient with P450c17 deficiency with unexpected pubic hair, breast development, and bilateral seminoma. Clinical Case: A 16 yrs-old female born to non-consanguineous parents was evaluated for primary amenorrhea. She had normal blood pressure, BMI =27, B5P4 Tanner stage and lack of axillary hair. The external genitalia presented a posterior labial fusion, a single perineal opening and a palpable right inguinal mass. Her karyotype was 46,XY. Basal LH levels were slightly elevated (12.8 IU/L), whereas FSH (9.1 IU/L) and ACTH (26 pg/mL) were normal. Elevated progesterone (P) and 17OHP levels were found before (1.4 and 15.7 ng/mL) and after ACTH stimulation (2.7 and 11.1 ng/mL). Basal cortisol levels were normal (12 ug/dL) without increment after ACTH. The hCG test (6000 IU) showed increased levels of P (2.5 ng/mL) and 17OHP (13.3 ng/mL). Basal and hCG stimulated E2 levels were surprisingly elevated (37 to 122 pg/mL) considering the low unchanged levels of testosterone (12 ng/dL) and androstenedione (<0.3 ng/mL) after hCG stimulation. Gonadectomy was performed and histological analysis revealed bilateral seminoma surrounded by Sertoli and Leydig cells presenting enlarged cytoplasm with eosinophilic granules. The compound heterozygous CYP17 mutation p.W406R/R358Q was identified in the patient. Discussion: We described the first report of a 46,XY DSD female due to CYP17 mutation with bilateral seminoma associated with atypical phenotype: full breast and almost complete pubic hair development associated with increase E2 secretion in the presence of very low androgenic levels. Evidences indicate that E2 exerts a permissive effect on pubic hair development, even in the presence of low circulating androgens. Rarely, 46,XY P450c17 deficient patients present gynecomastia and this is apparently associated with E2/P imbalance in breast tissue (3). Conclusion: The intriguing source of E2 in this patient has not been elucidated and might be associated with the presence of gonadal tumor.

- (1) Costa-Santos M et al., J Clin Endocrinol Metab 2004; 89:49-60
- (2) Brooke AM et al., J Clin Endocrinol Metab 2006; 91:2428-2431
- (3) Turan S et al., Eur J Endocrinol 2009; 160:325-330

Nothing to Disclose: LSH, EMFC, AZM, FMC, SD, BBM

Pub # P2-728

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Pediatric Endocrinology: Turner Syndrome, Growth, Pituitary, Congenital Adrenal Hyperplasia & Adrenal (1:30 PM-3:30 PM)

Title Novel Heterozygous Mutation in the Star (Steroidogenic Acute Regulatory Protein) Gene in a 46,XY Patient with Congenital Lipoid Adrenal Hyperplasia (CLAH)

Author String MS Baquedano, G Guercio, D Napoli, M Costanzo, E Berensztein, R Marino, M Bailez, E Vaiani, N Saraco, MA Rivarola, A Belgorosky
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Body StAR is required for adrenal/steroidogenesis and for male sexual differentiation. Recessive StAR mutations causes classic and nonclassic CLAH. StAR moves cholesterol from the outer (OMM) to the inner mitochondrial membrane, to be converted to pregnenolone. It is proposed that StAR acts on the OMM prior to import, must undergo a structural transition to exert activity, and phosphorylation of Ser195 augments its steroidogenic activity. We are reporting a 46,XY patient with ambiguous genitalia, primary adrenal insufficiency and severe impairment of adrenal and gonadal steroidogenesis. Genetic analysis revealed a novel heterozygous de novo IVS1-2A>G mutation in StAR gene and the pGly146Ala heterozygous polymorphism in SF1 gene. RT-PCR and sequence analysis from patient's testicular RNA showed an abnormal mRNA transcript of 397bp, corresponding to exon 2 skipping, as well as the wild-type (WT) 511bp transcript. Functional in vitro analysis was performed in COS cell transfected with the mutant and the WT (1) plasmids. Western blot analysis detected both the 37kDa precursor and 30kDa mature form. Immunofluorescence studies showed almost no colocalization of mitochondria and mutant protein (delta22-59StAR). StAR activity was measured as pregnenolone production in COS cells cotransfected with the cholesterol side-chain cleavage system (2). Delta22-59StAR activity was 65% ($\pm 13\%$) of WT. Cotransfection with WT and delta22-59StAR vectors also reduce WT activity by 62% ($\pm 13,9\%$). We identified a novel intronic heterozygous StAR mutation (IVS -2A>G) in a patient affected with CLAH. It produced a -exon2 aberrant mRNA resulting in the in-frame loss of amino acids 22 to 59 in the N-terminal mitochondrial targeting signal. It is reported that deletions of 62 amino-terminal residues of StAR precluded the mitochondrial entry, but it did not affect its activity (1). However, even though delta22-59StAR almost did not localize to the mitochondria, it showed a reduced activity. We speculated that aberrant folding may have compromise its ability to stimulate steroidogenesis. The delta22-59StAR might interfere with WT StAR by competing cholesterol binding causing an autosomal dominant form of StAR deficiency, explaining thereby the clinical phenotype in heterozygosis. Finally, StAR would require interaction with several other proteins on the OMM; associated mutations in some of them, which may have contributed to the severe clinical phenotype in our patient, could not be ruled out.

1- Bose HS, Lingappa VR, Miller WL. Rapid regulation of steroidogenesis by mitochondrial protein import. *Nature* 417:87-91, 2002.

2- Harikrishna JA, Black SM, Szklarz GD, Miller WL. Construction and Function of fusion enzymes of human cytochrome P450scc system. *DNA Cell Biol* 12:371-379, 1993.

Acknowledgments
We thanks Dr. W Miller for providing the F2 and wild type StAR plasmid and Dr. J Strauss III for providing the anti-human StAR serum.

Nothing to Disclose: MSB, GG, DN, MC, EB, RM, MB, EV, NS, MAR, AB

Pub # P2-729

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Pediatric Endocrinology: Turner Syndrome, Growth, Pituitary, Congenital Adrenal Hyperplasia & Adrenal (1:30 PM-3:30 PM)

Title Mineralocorticoid Replacement in Salt-Wasting Form of CAH Due to 21-Hydroxylase Deficiency during Early Infancy

Author String LG Gomes, TASS Bachega, G Madureira, BB Mendonca
Universidade de São Paulo, São Paulo, Brazil

Body Optimal glucocorticoid (GC) and mineralocorticoid (MC) replacements in salt wasting 21-hydroxylase deficiency (SW-21OHD) are crucial for adequate growth and neurological development. Directions of GC replacement in children are well established; however, the actual recommendation for MC replacement is general and suggests adjusting the dose individually, without a special recommendation during infancy, period in which many children have several dehydration episodes. Objective: To determine the appropriate fludrocortisone dose schedule in a group of SW-21OHD children adequately followed during early infancy. Methods: Twenty-three SW-21OHD patients, who presented good anthropometric and hormonal control, were evaluated retrospectively. Exclusion criteria were adrenal crisis and GC or MC side effects during the follow-up period. Patients were initially treated with cortisone acetate (CA) (~18 mg/m²/d), fludrocortisone (100-200 mcg/d) and salt supplementation (2 g/d). Assessments of fludrocortisone doses, clinical parameters (height, weight and blood pressure) and biochemical levels (sodium and potassium) were performed weekly during the first two months of life, monthly from 3 to 6 months and then every 3 months until 2 years of age. Testosterone, androstenedione and plasm renin activity (PRA) levels were measured every 3 months. Androgens levels aimed to be kept into normal range for sex and age and PRA into the upper-limit of normal range. Results: Fludrocortisone needs decreased overtime, the dose in the first semester was significantly higher than in the second semester, which in turn was significantly higher than in the second year of life (p<0.01). Mean fludrocortisone dose was 200 mcg at 0-6 months, 150 mcg at 7-18 months and 125 mcg at 19-24 months of life. CA dose was stable during these first 2 years (mean 16.8 mg/m²/d, SD 3.3). Serum sodium and potassium levels were normal during follow up, except in the first month of life (mean Na⁺ 132 and K⁺ 5,7 mEq/L), period of MC dose adjustment. Conclusions: MC needs in SW-21OHD decreased progressively during the first 2 years of life, confirming a partial aldosterone resistance during early infancy. Based on this retrospectively analysis of children followed homogeneously in a single Center, our study proposes a safety schedule for mineralocorticoid replacement during this critical developmental period.

Sources of Research Support: FAPESP # 05/04726-0, CNPq # 305117/2009-2.

Nothing to Disclose: LGG, TASSB, GM, BBM

Pub # P2-730

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Pediatric Endocrinology: Turner Syndrome, Growth, Pituitary, Congenital Adrenal Hyperplasia & Adrenal (1:30 PM-3:30 PM)

Title Adrenal Insufficiency and Other Neuroendocrine Dysfunctions in Septic and Non-Septic Acute Critical Illness and in the Early Post-Surgery Phase: A Comparison of Three Different Pediatric Groups

Author String G Karaguzel, S Atay, M Imamoglu, O Deger, A Okten
Karadeniz Technical University School of Medicine, Trabzon, Turkey; Karadeniz Technical University School of Medicine, Trabzon, Turkey; Karadeniz Technical University School of Medicine, Trabzon, Turkey; Karadeniz Technical University School of Medicine, Trabzon, Turkey

Body
Objective: To investigate adrenal insufficiency (AI) using low-dose corticotropin stimulation test (LDST) and other neuroendocrine dysfunctions in septic and nonseptic critically ill children, and in children at the early post-surgery phase.
Patients and Methods: Children with acute critical illness (ACI, n: 50) were consequently recruited the study. Of those 50 patients, 23 were with severe sepsis or septic shock (Group 1) and 27 with nonseptic patients (Group 2). Additionally, 24 children who underwent a major surgery were enrolled as Group 3. Basal serum levels of cortisol, corticotropin (ACTH), thyroid stimulating hormone (TSH), free triiodothyronine (FT3), free thyroxine (FT4), parathyroid hormone, dehydroepiandrosterone sulfate, glucose, albumin, calcium procalcitonin (PC), IL-6, TNF- α were measured. Subsequently, a LDST (1 [micro]g) was performed and ACTH and cortisol levels were repeated 3 days interval during the hospitalization.
Results: Group 1, Group 2 and Group 3 patients did not differ in gender and mean age. The patients in Group 1 had lower blood pressure than the patients in Group 2 and 3 ($p < 0.05$). The frequency of fluid and catecholamine refractory shock were higher in Group 1. Baseline and stimulated cortisol levels were 34.8 [micro]g/dl and 54.2[micro]g/dl in Group 1, 33.5[micro]g/dl and 49.4[micro]g/dl in Group 2, and 36.1[micro]g/dl and 45.0[micro]g/dl in Group 3 ($p > 0.05$). The incidence of AI was 17% in Group 1, 25% Group 2, and 41% in Group 3 ($p > 0.05$). The frequency of hyperglycemia was higher (59%) in Group 3 ($p < 0.05$). The frequency of hypocalcemia was 22%, 11%, and 4% in Group 1, 2 and 3, respectively. The levels of FT3, FT4, and TSH were not different between the groups and 29% of the patients had nonthyroidal disease. A positive correlation was found among the levels of cortisol and glucose and disease severity scoring. IL-6 and PC levels were correlated positively with basal cortisol levels in Group 1.
Conclusions: Although the incidence of AI depends on the criteria used to make the diagnosis, AI is common in children with ACI and early post-surgery phase. Very high levels of cortisol are likely to be associated with high risk of mortality. Being the highest frequency of hyperglycemia in Group 3 shows the importance of glucose monitorization besides cortisol at the early post-surgery phase. The frequency of other neuroendocrine dysfunctions are also high in children with septic and nonseptic ACI and in the early post-surgery phase.

Nothing to Disclose: GK, SA, MI, OD, AO

Pub # P2-731

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Pediatric Endocrinology: Turner Syndrome, Growth, Pituitary, Congenital Adrenal Hyperplasia & Adrenal (1:30 PM-3:30 PM)

Title Can Recovery from Hypothalamic-Pituitary-Adrenal (HPA) Axis Suppression Following Supraphysiological Doses of Glucocorticoids Be Predicted?

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Body
Background. Supraphysiological doses of glucocorticoids (GC) may cause adrenal insufficiency, which can be lethal if left unrecognized or untreated.
Objective. To determine which biomedical variables (8am serum cortisol, peak cortisol and [Delta] cortisol level after ACTH stimulation) and clinical variables (patient and pretest factors) are important for predicting recovery of the HPA axis.
Design/methods. We included patients who underwent low dose ACTH (1[μ g] testing between October 2008-June 2010 after receiving supraphysiological doses of GCs.
Results. Data from 104 patients were analyzed (age 8.4 \pm 5.4yrs, median 8.0; 57 girls). Baseline cortisol on the day of testing was normal (\geq 193nmol/L) in 77 pts. Sixty-three patients (57%) had a normal response to ACTH stimulation (peak cortisol \geq 500nmol/L). Peak cortisol was not correlated to baseline cortisol, duration of treatment or cumulative dose of GC; a normal baseline cortisol did not preclude subnormal response to ACTH (21%).
While clinical signs of hypoadrenalism were rare (n=2), the following were observed: growth deceleration (n=38; 37%), excessive weight gain (n=34; 33%), underlying disease (asthmatic n=30, dermatologic n=19, gastroenterological n=7, hematological n=22, nephrological n=1, oncological n=6, rheumatological n=16, cardiological n=3); duration of treatment (608 \pm 770days; median 370; 5-4226), maximal daily and cumulative oral or iv dose of GC in hydrocortisone equivalent, respectively (427 \pm 693mg/m²/day; median 200; 12-3700: 27084 \pm 35974mg/m²; median 15869; 82-178209); timing since last dose of GC (105 \pm 253days; median 46.5; 1-1584) and duration of physiological replacement with GC (215 \pm 255days; median 118; 0-1089).
Conclusion. Following treatment with suppressive doses of GC, a normal 8 am cortisol level is not helpful for predicting response to low dose ACTH stimulation. Given the absence of positive clinical and biomedical predictors, physicians must continue to screen patients with ACTH testing and maintain a state of vigilance for the risk of acute adrenal failure under situations of stress.

Nothing to Disclose: SW-R, JD, CB, CD, GVV, NA, CH

Pub # P2-732

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Pediatric Endocrinology: Turner Syndrome, Growth, Pituitary, Congenital Adrenal Hyperplasia & Adrenal (1:30 PM-3:30 PM)

Title *CYP21A2* Genotypes Do Not Predict the Severity of Hyperandrogenic Manifestations in Non-Classical Form of 21-Hydroxylase Deficiency

Author String VO Moura, LG Gomes, JAM Marcondes, BB Mendonca, TASS Bacheга
Faculdade de Medicina, Universidade de São Paulo, São Paulo, Brazil

Body **Background:** NCAH is a frequent disorder caused by *CYP21A2* mutations. The genotypes carry mutations predicting moderate enzymatic activity impairment in homozygosis (C/C) or in compound heterozygosis with those predicting severe impairment (A/C). NCAH has a wide hyperandrogenic phenotypic variability and there is scarce data evaluating correlations between severity of hyperandrogenic signs and genotypes. **Objective:** To analyze the correlation between severity of hyperandrogenic phenotypes and genotypes in NCAH patients. **Patients and Methods:** Out of 175 patients with ACTH-17OHP levels >10 ng/mL, we selected 112 (106 F) who had mutations identified in both *CYP21A2* alleles by sequencing/MLPA analysis. Genotypes were classified according to severity: C/C moderate (n= 55) and A/C severe (n= 57) and compared with age at diagnosis, hyperandrogenism severity and serum hormonal levels. Parametric or nonparametric tests were used in the statistical analysis as appropriate. **Results:** In children with precocious pubarche, 30 carried the A/C and 21 the C/C genotypes and among adults, 23 carried the A/C and 27 the C/C genotypes. There was no difference between mean age at diagnosis between A/C and C/C genotypes (children 6 ± 2.5 yrs old *versus* 7 ± 2.3 yrs old, respectively and adults 29 ± 11 yrs old *versus* 28 ± 13 yrs old, respectively, $P > 0.05$). Eleven patients were asymptomatic, diagnosed in familial studies, 5 carried the A/C and 6 the C/C genotypes. Among adult females, 49 (90%) had hirsutism and surprisingly, higher Ferriman scores were observed in the C/C group (17 ± 7) than in the A/C group (12 ± 5) ($P = 0.04$). There was no difference in the frequency of hirsutism with/without menstrual abnormalities as well as of infertility between A/C and C/C groups. Three children and 6 adults had slight clitoromegaly, 6 carried the A/C and 3 the C/C genotypes. Basal 17OHP levels were higher in A/C (21 ± 47 ng/mL) than in C/C groups (9 ± 10 ng/mL) ($P = 0.001$), similar to that which occurred with ACTH-17OHP levels (A/C 59 ± 42 and C/C 39 ± 22 ng/mL, $P = 0.009$). In contrast, testosterone levels did not differ between adult symptomatic females with A/C (74 ± 49 ng/dL) and C/C groups (64 ± 51 ng/dL) ($P > 0.05$). **Conclusion:** in this large cohort, we confirmed the strong correlation among genotype, nonclassical form and 17OHP levels. On the other hand, NCAH genotypes did not predict hyperandrogenic sign severities, which may depend on individual peripheral androgen sensitivity.

Sources of Research Support: FAPESP # 08/51624-6, FAPESP # 05/04726-0, CNPq For TASSB #305117/2009-2.

Nothing to Disclose: VOM, LGG, JAMM, BBM, TASSB

Pub # P2-733

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Pediatric Endocrinology: Turner Syndrome, Growth, Pituitary, Congenital Adrenal Hyperplasia & Adrenal (1:30 PM-3:30 PM)

Title Bone Age Advancement Due to Androgen Exposure Does Not Impair the Normal Adult Target Height in Children with Functioning Adrenocortical Tumors during Early Infancy

Author String TC Goncalves, S Domenice, AAL Jorge, AM Lerario, MQ Almeida, AC Latronico, MAA Albergaria, BB Mendonca, MCV Fragoso
Hospital das Clinicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil

Body **Background:** Exposure to androgen excess during a child's growth may compromise adult height due to a precocious skeletal maturation and epiphyseal fusion. The majority of patients with congenital adrenal hyperplasia without good control are short in adulthood. Virilizing adrenal cortical tumors (ACTs) are an additional disorder that can expose children in early infancy to high levels of androgens. However, the impact on final height is unknown. **Objective:** To analyze the growth pattern, bone age advancement and adult height of children treated for functioning ACT: virilizing tumors (V) and mixed (M) androgen and glucocorticoid secreting tumors. **Subjects:** Twelve patients (4 males and 9 females) with functioning ACTs and followed at the Endocrinology Division at Hospital das Clinicas da Universidade de Sao Paulo were evaluated. All patients showed clinical signs of virilization, and in six of them, absence of cortisol suppression >1.8 [μ] g/dL) was demonstrated after overnight low dose of dexamethasone suppression test (vo, 10 [μ]g/Kg). After surgical treatment, all patients maintained androgen levels at the pre-pubertal range except the patient that developed asynchrony adrenal tumor, requiring a second surgical approach. These patients were followed for 13 to 26 yrs and none of them developed central precocious puberty. All of them reached final height within adult bone age. **Results:** The final height of these patients was within their individual target height range ($TH \pm 1.5$ SD) except the patient 9 with - 2.2 standard deviation to final stature. **Discussion:** Catch-down growth is a phenomenon seen in young children after a period of growth acceleration when the cause of growth stimulus is removed. This phenomenon was not observed after ACTs resection in the follow-up of these patients. Growth velocity was maintained high, especially in the first year after surgery, despite the presence of normal prepubertal hormonal levels. Their growth pattern might have helped them to achieve their target height. **Conclusion:** Although patients with virilizing ACT present at diagnosis an increased height and bone age, their height at adulthood is usually within the normal familial range. This retrospective study suggests that the exposure to androgen excess in early infancy for a short period of time does not significantly impair the achievement of their full genetic growth potential.

Sources of Research Support: In part by Grant Fundacao de Amparo a Pesquisa de Sao Paulo (FAPESP) # 09/51174-3.

Nothing to Disclose: TCG, SD, AALJ, AML, MQA, ACL, MAAA, BBM, MCVF

Pub # P2-734

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Pediatric Endocrinology: Turner Syndrome, Growth, Pituitary, Congenital Adrenal Hyperplasia & Adrenal (1:30 PM-3:30 PM)

Title Overestimation of Final Height Prediction in Patients with Classical Adrenal Hyperplasia Using the Bayley & Pinneau Method

Author String W Bonfig, H Schmidt, HP Schwarz
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Body A typical growth pattern with decreased pubertal growth spurt of patients with CAH has been previously identified by us and other authors. Therefore final height prediction using the Greulich & Pyle and Bayley & Pinneau (B&P) method seems inappropriate for these patients.
Patients & Methods: Using growth data of 92 patients (57F/35M) with classical CAH due to 21-Hydroxylase deficiency (38 SV/54SW) final height prediction with the B&P method was compared to real final height. Subsequently new tables for final height prediction in patients with classical CAH were derived from the data
Results: In females mean final height was 159.9±5.3 cm (-1.0±0.7 SDS) compared to mean predicted final height of 167.9±10.7 cm (+0.5±1.7 SDS), p<0.001, overestimation 7.3±9.5 cm. At start of puberty (at the time of final height prediction) mean bone age was 10.5±1.5 years (with a mean chronological age of 10.0±1.7 years). Bone age was accelerated in 14 females, appropriate for chronological age in 38 females and retarded in 5 females.
In males mean final height was 170.1±6 cm (-1.2±0.8 SDS) compared to mean predicted final height of 185.6±13.4 cm (+1.2±1.9 SDS), p<0.001, overestimation 13.9±10.8 cm. At start of puberty (at the time of final height prediction) mean bone age was 11.7±1.6 years (with a mean chronological age of 10.5±2.0 years) Bone age was accelerated in 15 males, appropriate for chronological age in 18 males and retarded in 2 males. A final height prediction model for CAH patients was derived from these data.
Conclusion: In classical CAH final height prediction results in significant overestimation of final height when the B&P tables are used. New final height prediction tables were derived from a large group of homogeneously treated patients receiving traditional glucocorticoid and mineralocorticoid treatment.

Nothing to Disclose: WB, HS, HPS

Pub # P2-735

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Pediatric Endocrinology: Turner Syndrome, Growth, Pituitary, Congenital Adrenal Hyperplasia & Adrenal (1:30 PM-3:30 PM)

Title Final Height of Subjects with Non-Classical 21-Hydroxylase Deficiency by Age at Initiation of Glucocorticoid Therapy and Genotype

Author String O Eyal, Y Tenenbaum-Rakover, S Shalitin, N Weintrob
Dana Children's Hospital, The Tel Aviv Sourasky Medical Center, Tel Aviv, Israel; Ha'Emek Medical Center, Afula, Israel; Schneider Children's Medical Center of Israel, Petah Tikva, Israel

Body **Background:** Non-classical 21-hydroxylase deficiency (NC21OHD) is a mild form of congenital adrenal hyperplasia (CAH) associated with different degrees of postnatal virilization developing from infancy to adulthood. The genotype might be either homozygous or compound heterozygous for mild mutations or compound heterozygous for one mild and one severe mutation of the gene encoding 21-hydroxylase (*CYP21*). It has been shown that subjects with classical form of CAH tend to be shorter than expected from their midparental height. A recently published meta-analysis of 35 studies showed that patients with CAH achieve final height of about 1.4 standard deviations below the mean height and -1 SDS below midparental height SDS (1).
Aims: 1. to determine whether NC21OHD compromises final height. 2. to look for clinical parameters affecting final height in this population.
Methods: Retrospective review of medical records of subjects with NC21OHD who have reached final height for age at diagnosis, age at initiation of therapy, midparental height and *CYP21* genotype. Diagnosis was made according to measurements of the 17-hydroxyprogesterone response to intravenous synthetic ACTH. The SD score (SDS) for final height and corrected height SDS (defined as final height - midparental height SDS) were determined for each subject.
Results: Final height was available for 104 patients (81 females) diagnosed at mean age of 10.5 (median 8.5, range 0.1-32.1 years). Genotype was available for 86 patients of whom 60 (70%) were homozygous for V281L or compound heterozygous for mild mutations (group A), 17 (20%) were compound heterozygous for one mild and one severe mutation (group B), and 9 (10%) were heterozygous for V281L (group C). The mean final height SDS achieved by the entire study population was -0.55 ± 1 , and the mean corrected height SDS was -0.16 ± 0.7 . Patients in group A achieved mean corrected height SDS of -0.16 ± 0.7 , compared to patients in group B who achieved mean corrected height SDS of -0.34 ± 0.7 . No significant correlation or association was found between corrected height SDS and genotype, gender, or age at diagnosis, although those with one severe mutation tend to be shorter. **Conclusions:** Unlike classical CAH NC21OHD does not seem to affect final height significantly irrespective of clinical parameters such as gender, age at diagnosis, genotype or treatment. This result may affect our clinical approach to this population.

(1) Muthusamy K et al., J Clin Endocrinol Metab 2010; 95:4161

Nothing to Disclose: OE, YT-R, SS, NW

Pub # P2-736

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Pediatric Endocrinology: Turner Syndrome, Growth, Pituitary, Congenital Adrenal Hyperplasia & Adrenal (1:30 PM-3:30 PM)

Title Carrier Frequency of Congenital Adrenal Hyperplasia (21-Hydroxylase Deficiency) in Korean Children with the Majority of Early Puberties

Author String PS Oh
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Body

Aims
Nowadays, many Koreans are concerned with the phenomenon of early puberty. And I know a few reports of congenital adrenal hyperplasia (CAH) carrier frequency in some populations. So, prospectively I scheduled to know the CAH (21-hydroxylase deficiency) carrier frequency in Korean children with the majority of early puberties.

Methods
I started to gather the study group since July, 2008. ACTH stimulation tests have been done for 171 Korean children (female 161, male 10, CA: 8.25 ± 1.44 , min 3.44~max 12.78 yr, BA advanced: 1.68 ± 0.83 , min -0.71~max 3.72yr) with the majority of early puberties and the results of 17-OH-progesterone (17OHP) and cortisol have been analyzed with bone age etc. Rationale: In congenital adrenal hyperplasia, the synacthen test is useful in diagnosing milder or rare enzyme blocks by examining ratios of various adrenal steroids to their precursor compounds. The commonest ratio examined is that of 17OHP/cortisol in suspected non-classical or simple virilizing CAH or the heterozygote state (Based on Australian Council of Healthcare Standards Guidelines). Normal ratio of 17OHP to cortisol at 30 mins < 0.023 , Ratios up to 0.08 suggest heterozygosity for 21-hydroxylase deficiency and Ratios > 0.1 suggest CAH (21-hydroxylase deficiency).

Results
I found out 25 heterozygote CAH carriers in the study group. And I could find an interesting aspect of 17OHP results. Almost always the cortisol responses after ACTH stimulation were elevated, but in some cases the 17OHP responses were not. In many children, 17OHP were not sufficiently elevated ($60/171 = 36\%$), or even the same or decreased responses ($34/171 = 20\%$) could be seen among them.

Conclusions I think that the heterozygote CAH (21-hydroxylase deficiency) carrier frequency would be about 14.62% in Korean children with the majority of early puberties. And it seems that another mechanism (s)/pathway(s) of ACTH stimulation for cortisol could be present.

Nothing to Disclose: PSO

Pub # P2-737

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Pediatric Endocrinology: Turner Syndrome, Growth, Pituitary, Congenital Adrenal Hyperplasia & Adrenal (1:30 PM-3:30 PM)

Title Ciproterone Acetate Therapy Improves Final Height Achievement in CAH Patients

Author String JRZ Zucare, H Nicolielo, RPP Moreira, LG Gomes, VN Brito, BB Mendonca, TASS Bacheга
Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil

Body Background: The treatment of congenital adrenal hyperplasia (CAH) is based on short half-life glucocorticoid (GC) regimens with the purpose of preventing virilization and allowing normal growth. With this therapy there is a difficult balance between hyperandrogenism and hypercortisolism, which can affect final height (FH). Despite an adequate replacement therapy, CAH patients could present FH <1.5 SD of the normal population. Ciproterone acetate (CP) is an antiandrogen drug with glucocorticoid activity. CP could act synergistically with GC blocking bone maturation in CAH. However, the CP effect on FH of CAH patients has never been previously evaluated. Objective: to analyze the impact of CP on final height achievement in CAH. Patients and Methods: 7 patients (4F), 4 with simple viriling (SV) and 3 with nonclassic (NC) forms. Mean chronological age (CA) at CAH diagnosis was 5.6 ± 4 yrs old and cortisone acetate $18 \text{ mg/m}^2/\text{d}$ was introduced. Mean CA and bone age (BA) at the beginning of CP therapy was 8.7 ± 1.7 and 11.6 ± 1.3 yrs old, respectively and the mean SD of height adjusted for BA was -1.5 ± 0.85 . All patients were prepubertal. CP was started at mean doses of $75 \text{ mg/m}^2/\text{d}$ and subsequently GC doses were adjusted. We aimed to maintain testosterone levels $<14 \text{ ng/dL}$ and androstenedione levels $<2 \text{ ng/mL}$, without suppression of 17OHP levels. Height was described as SD and predicted FH was evaluated by average Bayley Pinneau method. Paired t-test was used in the statistical analyses. Results: The duration of CP therapy was 3.3 ± 1 yrs and mean BA advancement was 1.4 ± 1.6 yrs. Mean CP dose was $77 \pm 29 \text{ mg/m}^2/\text{d}$ and mean GC dose decreased to $12 \pm 15 \text{ mg/m}^2/\text{d}$. The mean height gain during CP therapy was 15 ± 6 cm and after the end of therapy was 12 ± 2 cm. The mean predicted FH at the end of CP therapy was higher than predicted at the start (161.6 ± 25 and 153.4 ± 16.6 cm, respectively). The achieved FH was higher than initially predicted ($P=0.002$; power of performed test 0.99). There was no difference between mean FH and mean target height (TH) (165 ± 25.8 and 166 ± 20.6 , respectively, $P>0.05$). Mean SD of FH was -0.44 ± 0.87 and mean SD of TH was -0.23 ± 1.9 . Except for weight gain in 2 patients, no adverse effects were observed. Conclusions: Ciproterone acetate therapy was efficient in controlling BA maturation during GC treatment of CAH. Moreover, ciproterone acetate therapy restores the growth potential for achievement of FH within the normal range of TH.

Sources of Research Support: CNPq # 305117/2009-2, FAPESP #05/04726-0 and #09/54238-2.

Nothing to Disclose: JRZZ, HN, RPPM, LGG, VNB, BBM, TASSB

Pub # P2-738

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Pediatric Endocrinology: Turner Syndrome, Growth, Pituitary, Congenital Adrenal Hyperplasia & Adrenal (1:30 PM-3:30 PM)

Title Experience of Congenital Adrenal Hyperplasia in Eastern India from 1996 to 2010

Author String A Maiti, S Chatterjee
NRS Medical College, Kolkata, India; Park Clinic, Kolkata, India

Body Aims: The conversion of cholesterol to cortisol takes place through 5 enzymatic steps. Mutations causing deficiency of any of these enzymes result in a group of conditions called Congenital Adrenal Hyperplasia (CAH). Data regarding presentation, course and outcome of patients with CAH is scarce in South-Asia. It is possible that South Asian patients behave differently from Caucasian patients with CAH. We therefore collated our experience with CAH and compared it with results from other centres in the south Asian region and elsewhere. Methods: This retrospective study analyzed the records of 86 CAH patients who were seen at a tertiary care referral centre in Eastern India from 1996 to 2010. Results: Of these 86 patients, 45 (52.3%) had classic disease (C CAH) and 41 (47.7%) had non-classic disease (NC CAH). In the C CAH group, 18 (20.9%) had salt wasting CAH (SW CAH) while 27 (31.4%) had simple virilising disease (SV CAH). Ten (11.6%) had a 46,XY karyotype. Patients with SW CAH were diagnosed earlier in infancy at a median age of 0.5 years, than those with SV type, who were diagnosed at 9 years ($p<0.05$). The NC CAH patients were diagnosed still later, at 20.4 years. In patients with SW CAH, boys were diagnosed at an earlier median age of 0.1 years than girls who were diagnosed at 6.4 years ($p<0.05$). Sixteen girls (21% of the girls) underwent surgery and while 12 had vaginoplasty alone, 4 had vaginoplasty and clitoroplasty. One needed bilateral laparoscopic adrenalectomy to control her symptoms. One NC CAH patient had a successful pregnancy with clomiphene stimulation. Primary autoimmune hypothyroidism, found in 7 (8.1%) was the commonest coexisting abnormality. Conclusion: The skewed male: female ratio suggested that a substantial proportion of males were being missed and consequently may have died. The majority of the diagnosed patients ($n=55$, 64%) have been lost to follow-up. The remaining patients could be reasonably controlled in our setting with standard medical therapy.

Nothing to Disclose: AM, SC

Pub # P2-739

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Pediatric Endocrinology: Turner Syndrome, Growth, Pituitary, Congenital Adrenal Hyperplasia & Adrenal (1:30 PM-3:30 PM)

Title Prevalence of Congenital Adrenal Hyperplasia (CAH) Due to 21-Hydroxylase Deficiency in a Jordanian Sample Pool

Author String H Daggag, D Hiyosat, N Khawaja, M Abu-Jbara, A Al-Batayneh, M El-Khateeb, K Al-Ajlouni
National Center for Diabetes, Endocrinology and Genetics, Amman, Jordan

Body CAH represents a family of autosomal recessive disorders. About 95% of the CAH cases are caused by 21-hydroxylase deficiency, resulting from a defect in the *CYP21A2* gene. The main aim of the current study was to screen the Jordanian population for the 8 most common small *CYP21A2* mutations and introduce a prenatal and a neonatal genetic test for CAH in Jordan. Similar mutational analysis studies have been performed in other Arab countries such as Tunisia, Lebanon and Iran, however, this is a first comprehensive study of its kind, performed on the Jordanian population. Although no accurate population study exist, it is very clear that the incidence of CAH in Jordan is high (based on the high percentage of consanguineous marriages and high number of CAH patients being admitted to clinics nationwide). Current sample study consisted of 37 clinically diagnosed index patients, including 20 males and 17 females. In most cases, DNA samples for family members were also available, bringing the total number of samples up to 140. As a screening method we implemented the amplification refractory mutation system (ARMS), optimizing conditions for the 8 most common *CYP21A2* mutations (P30L in exon1, In2cs in intron 2, 8bp deletion in exon 3, I172N in exon 4, exon 6 cluster mutation, V281L in exon 7, Q318X and R356W in exon 8). ARMS analysis identified mutations in 33 index patients, 15 of which were heterozygotes for a single mutation, 5 were compound heterozygotes, 11 were homozygotes and 2 were compound homozygotes. Mutation frequency was the highest for In2cs mutation at 37.8%, followed by Q318X at 24.3% and 13.5% for 8bp deletion. Although this mutational spread was unique to the Jordanian population, the prevalence of the In2cs mutation was in agreement with the average world studies conducted in 2003 (1). It is important to also note that in 25% of CAH cases due to *CYP21A2* gene defect, mutations are caused by large gene deletions (i.e. 30 kb gene deletion). Hence, to be able to obtain a true statistical representation of *CYP21A2* mutations in the Jordanian population, our sample pool needs to be tested for the 30 kb deletion. This will be achieved through Multiple Ligation-dependant Probe Amplification (MLPA) method, utilizing a CAH-MLPA kit. Once the mutational analysis is complete, we can implement a prenatal and a neonatal testing on a national scale, hoping to reduce the overall incidence of CAH in a Jordanian population.

(1) Kotaska K, Lisa L, Prusa R. Common CYP21 gene mutations in Czech patients and statistical analysis of worldwide mutation distribution. Cent Eur J Public Health; 11: 124-128

Nothing to Disclose: HD, DH, NK, MA-J, AA-B, ME-K, KA-A

Pub # P2-740

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Pediatric Endocrinology: Turner Syndrome, Growth, Pituitary, Congenital Adrenal Hyperplasia & Adrenal (1:30 PM-3:30 PM)

Title A Homozygous Missense Mutation in SCNN1A Is Responsible for a Transient Form of Pseudohypoaldosteronism Type 1

Author String M Dirlewanger, D Huser, M-C Zennaro, E Girardin, L Schild, VM Schwitzgebel
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Body
Background: Pseudohypoaldosteronism type 1 (PHA1) is a monogenic disorder of mineralocorticoid resistance characterized by salt wasting, hyperkalemia, high aldosterone levels and failure to thrive. The multisystemic form is caused by mutations in the epithelial sodium channel ENaC and symptoms are severe and persisting. The renal form involves the mineralocorticoid receptor; symptoms are milder and usually transient.
Clinical case and results: Here we present the case of two siblings with a homozygous missense mutation of the SCNN1A gene (c.727T>C/p.Ser243Pro), encoding the α subunit of ENaC (α ENaC) with contrasting clinical manifestations. In the prematurely, first born boy we observed a severe salt-losing syndrome. On day sixteen of life he developed hyponatremia, hyperkalemia and metabolic acidosis and aldosterone was elevated. The diagnosis of PHA1 was made and Na⁺Cl⁻ substitution and cation exchange resin therapy instated, which normalized the electrolytes. The treatment could be stopped at six months of age. Two years later a brother was born at term. He also carried the homozygous Ser243Pro mutation, had biochemical alterations, but no clinical PHA1.
In vitro studies: In heterologous expression system, the Ser243Pro mutation leads to a partial loss of function of ENaC that is exacerbated in the presence of high intracellular and extracellular Na⁺ load. These in vitro results parallel our human observation, since ENaC function almost normalized in our patient during the postnatal period, once urinary Na⁺ concentration decreased. Furthermore, the younger sibling born at term, harboring the same homozygous S243P mutation, showed an even milder impairment of ENaC function.
Conclusion: The function of the mutated channel is decreased in situations of increased Na⁺ load, which explains the clinical manifestation in the prematurely born infant, which is associated with incomplete nephrogenesis. This report sheds a new light on the importance of ENaC in regulating Na⁺ excretion during the perinatal period in humans.

Sources of Research Support: Grant 31003A-120093 from the Swiss National Science Foundation (to L.S.).

Nothing to Disclose: MD, DH, M-CZ, EG, LS, VMS

Pub # P2-741

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Pediatric Endocrinology: Turner Syndrome, Growth, Pituitary, Congenital Adrenal Hyperplasia & Adrenal (1:30 PM-3:30 PM)

Title Cortisol Response to Low-Dose ACTH Stimulation Test in Non-Stressed Pediatric Patients

Author String J Sequeira, RA Noto, Q Shi, M Sandu, F Altunkaya, R Deen, A Romano
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Body **Objective:** To more clearly define normal cortisol response to a low dose 1 [mu]g ACTH challenge in non-stressed pediatric patients (pts).
Subjects: Our database of pts was reviewed for subjects who underwent a low dose ACTH stimulation test (LDA). 210 pts, termed the all patient group (APG), were found. Of these, 133 also underwent a GH stimulation test (GHS). 54 pts who passed the GHS were considered the control patient group (CPG).
Methods: For the LDA, pts received at 8am, 1 [mu]g Cortrosyn IV and blood was drawn at times 0, 20, 30, 40, 60 minutes for cortisol assessment. The GHS pts received L-dopa and arginine. Normal GH response was considered as any GH level [ge] 10 ng/dl. GH and cortisol levels were determined by Immulite kits. This study was IRB approved.
Results: CPG: Mean age 11.8 ± 3.5 years. 42.6% female; 57.4% male. The mean/median baseline cortisol level were 14.9 ± 6.1/13.2 [mu]g/dl. The mean/median peak cortisol level were 24.9±6.7/25.6 [mu]g/dl. The mean/median GH max level were 18.4 ± 6.9/15.7 ng/dl. The correlation between peak and baseline cortisol values was r = 0.54 (p<0.0001). The percentages of pts passing the LDA at peak cortisol cutoffs of 14, 15, 16, 17, 18, 19 and 20 [mu]g/dl were 96.3, 94.4, 92.5, 90.7, 87.0, 83.3, and 79.6% respectively. A false positive rate of 10% would be at a peak cortisol level of 17.4 [mu]g/dl.
APG: Mean age 11.4 ± 4.2 years. 43.3% female; 56.7% male. The mean/median baseline cortisol levels were 13.1 ± 7.2/11.8 [mu]g/dl. The mean/median peak cortisol level were 25.2 ± 8.1/24.9 [mu]g/dl. The correlation between peak and baseline cortisol values was r = 0.44 (p<0.0001). The predictive value of a baseline cortisol predicting a peak cortisol of [ge] 17[mu]g/dl at baseline cortisol of 7, 8, 9, and 10 [mu]g/dl is 94.8, 95.1, 97.3 and 97.0% respectively.
Discussion: Utilizing 10% as an acceptable false positive rate, a cutoff of 17 [mu]g/dl seems to be ideal for representing a normal cortisol response to LDA. The 3 CPG pts with cortisol responses of 14 - 16.9 [mu]g/dl could be considered to have borderline normal responses. The 2 CPG pts with cortisol values <14 [mu]g/dl should be considered false positives. For the APG and CPG, there was a good correlation between baseline and peak cortisol levels. According to our data, a baseline cortisol of 9 [mu]g/dl has the best predictive value of a normal response to LDA. In conclusion, a baseline cortisol of 9 [mu]g/dl and a peak cortisol of 17 [mu]g/dl on LDA suggest normal adrenal function in children.

Nothing to Disclose: JS, RAN, QS, MS, FA, RD, AR

Pub # P2-742

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Pediatric Endocrinology: Turner Syndrome, Growth, Pituitary, Congenital Adrenal Hyperplasia & Adrenal (1:30 PM-3:30 PM)

Title Growth and Adult Heights among Four Groups of Males Treated with Gonadotropin-Releasing Hormone Analog during Puberty

Author String AK Goldyn, J Fuqua, E Kalaitzoglou, C Houk, PA Lee
Indiana University School of Medicine Riley Hospital for Children, Indianapolis, IN; Hershey Medical Center, Hershey, PA; Medical College of Georgia, Augusta, GA

Body

Introduction: Gonadotropin-releasing hormone analogues (GnRHa) are the treatment of choice for central precocious puberty (CPP) to delay pubertal development in order to maintain growth potential. GnRHa are also used in an attempt to increase adult height among short individuals who enter puberty at the usual age. However, adult height (AH) outcome data are limited for males.

Objectives: To assess the efficacy of GnRHa in both males with CPP and in pubertal-aged males with short stature and predicted adult heights below the normal range.

Methods: Medical records were reviewed for 51 males treated with GnRHa for whom AHs were available. Patients were divided into 4 groups: 1) CPP without other diagnoses [n=17], 2) CPP with concomitant diagnoses impacting growth [n=9], 3) Idiopathic/familial short stature at onset of normally-timed puberty [n=8] and 4) Short stature at the onset of normally-timed puberty with other diagnosis impacting growth [n=17]. Puberty and suppression during therapy were documented by Tanner staging, growth rates and hormone levels including responses to GnRHa stimulation testing. Outcome data included height, bone age (BA), adult height, and target height (TH).

Results: Among those with CPP alone (Group 1), 12 of 17 had adult heights > -2 standard deviations (SD). Two of 5 with AHs <-2SD were within 5 centimeters (cm) of their TH. In Group 2, 1 of 9 had an AH > -2SD with no individuals within 5 cm of TH. Four had height plotted for BA < -2SD at onset of therapy. Outcomes in Group 3 include 3 of 8 with normal AHs. Three individuals with AHs <-2SD had TH SD scores <-2SD. In Group 4, 7 of 17 had AHs within the normal range and 1 had AH within TH range. Twelve of 17 were GH deficient patients entering puberty at a normal age with 3 having a height for BA <-2SD at therapy initiation. AHs were in the normal range for 6; the other 6 were shorter for age and had more advanced BAs at onset of therapy. Normal AHs were associated with early GnRHa initiation during puberty when BAs were <14 years along with growth rates of 4-5 cm/year, and minimal BA progression during therapy.

Conclusions: Among males with CPP, timely therapy results in AHs within the normal range. Among the other groups, outcomes varied. Those with severe short stature (Ht SD<-2.5) and advanced bone ages (>14 years) gain little with therapy.

Disclosures: JF: Advisory Group Member, Pfizer, Inc.; Consultant, Abbott Laboratories, Ipsen. PAL: Ad Hoc Consultant, Abbott Laboratories; Study Investigator, Novo Nordisk, Lilly USA, LLC, Abbott Laboratories, Pfizer, Inc. Nothing to Disclose: AKG, EK, CH

Pub # P2-743

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Pediatric Endocrinology: Turner Syndrome, Growth, Pituitary, Congenital Adrenal Hyperplasia & Adrenal (1:30 PM-3:30 PM)

Title Evaluation of the Bayley-Pinneau Method of Adult Height Prediction and Variables That Interfere with Its Accuracy in Idiopathic Short Children (ISS) or Children Born Small for Gestational Age (SGA)

Author String CN Lauretti, TC Martins, IJP Arnhold, BB Mendonca, AAL Jorge
Disciplina de Endocrinologia, Hospital das Clinicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil

Body **Background:** Predicted final height provides valuable data toward identification of short stature children that might benefit from treatment with recombinant growth hormone.
Objective: To identify the clinical features that can affect the prediction of final height by BP methods in children with ISS or born SGA.
Material and Methods: 34 patients (12 female, 22 male) with idiopathic short stature (n = 21) or born small for gestational age (n = 13) were followed without treatment, from infancy until final height (mean follow-up period of 6 yr). Exclusion criteria were growth hormone deficiency, chromosome disorders, malformation syndrome, skeletal disorders, chronic diseases and steroid therapy. For each patient, three bone age assessments (Greulich-Pyle) and final height prediction (Bayley-Pinneau) were obtained. At the first evaluation chronological age was 11 ± 2.5 y, bone age was 8.7 ± 2.4 y and height SDS was -2.9 ± 0.8 . The sensitivity and specificity to predict short stature in adulthood (height SDS < -2) were calculated. The variables that may interfere with the BP accuracy were identified by multiple linear regressions.
Results: There was a positive correlation between BP predicted final height and real final height ($r = 0.76$, $p < 0.001$). The final height was consistently lower than predicted height (mean difference of 5.6 cm, 95%CI of 2.6 to 8.5 cm). Final height prediction was more accurate in female patients ($p = 0.006$), prepubertal patients ($p = 0.021$) and in children with higher BP height prediction ($p < 0.001$). Higher BMI and lower bone age were the factors that caused greater error in the height prediction. There was a tendency to a lower accuracy of BP to predict the correct adult height in SGA children than ISS children ($p = 0.06$). Of the 34 children followed, 22 (64.7%) had final height below the normal range. The BP method had a sensitivity of 57% (95% CI 34 to 78%) and specificity of 100% (95% CI 74 to 100%) in predicting short stature at adult age. There was no significant accuracy difference in the BP-height prediction using only the first available bone age or the subsequent ones obtained during follow-up.
Conclusion: Bayley-Pinneau method had high diagnostic specificity to detect short stature in adulthood. Its accuracy in predicting final height is higher for girls and for patients with low BMI, higher bone age and height at baseline assessment. Generally, BP overestimates the value of final height.

Nothing to Disclose: CNL, TCM, IJPA, BBM, AALJ

Pub # P2-744

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Pediatric Endocrinology: Turner Syndrome, Growth, Pituitary, Congenital Adrenal Hyperplasia & Adrenal (1:30 PM-3:30 PM)

Title Absence of GH-Releasing Hormone (*GHRH*) Mutations in Selected Patients with Isolated GH Deficiency

Author String MM Franca, AAL Jorge, KS Alatzoglou, LR Carvalho, BB Mendonca, L Audi, A Carrasco, MT Dattani, IJP Arnhold
Hospital das Clinicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil; Institute of Child Health, London, UK; Institut de Recerca, Hospital Vall d'Hebron, Centre for Biomedical Research on Rare Diseases (CIBERER), Autonomous University, Barcelona, Spain

Body
Introduction: GHRH induces GH transcription and hormone release by acting on GHRH receptors (GHRH-R) expressed on the somatotroph cell surface. Anatomic and functional abnormalities of the connection between the hypothalamus and the anterior pituitary, which prevent interaction of GHRH with its receptor, are the most important causes of clinical growth hormone deficiency. Mice with knockout of the *Ghrh* gene showed significant post-natal growth retardation due to IGHD, as shown by reduced pituitary GH mRNA and protein content, reduced serum IGF-I, and reduced liver IGF-I mRNA. Therefore, *GHRH* is an obvious candidate for GH deficiency. Previous *GHRH* studies were published in the early 1990's and did not identify gene mutations, but used a less sensitive methodology compared to present techniques.
Objective: The aim of this study was to identify mutations in *GHRH* in a large cohort of patients with isolated GH deficiency (IGHD).
Patients and Methods: DNA was isolated from 156 patients with IGHD from national and international centers. This cohort was selected to include patients with either low peak GH after stimulation tests ([Ie]5 ng/ml, 94% of patients), positive family history (38%), and/or absence of mutations in *GHI* (86%) and/or *GHRH-R* (69%) The entire coding sequence and splice sites of *GHRH* were amplified by PCR with intronic primers followed by automatic sequencing.
Results: We identified 5 novel heterozygous allelic variants in 4 patients: c.147 C>T p.S49S, IVS1 -74 T>C, IVS3 -47 del1 and IVS3 +7 G>A /IVS3+41 G>A. One patient had the previously described polymorphism IVS1 -70 G>A. These variants do not predict change of amino acids or splice sites according to *in silico* studies. Additionally, c.223 C>T, p.L75F within *GHRH* was detected in heterozygosity in 5 patients (4 from Brazil). Interestingly, this variant had been reported in 16% of alleles from a Nigerian population, 9% in African Americans, none in Europeans and less than 1% from Asia. These data suggest an African origin for the GHRH p.L75F polymorphism in the Brazilian patients. No functional mutations were found in the present cohort.
Conclusions: *GHRH* mutations were not identified in a large selected cohort of patients with IGHD, suggesting that, if they exist, they may be an extremely rare cause of IGHD. Other, as yet unidentified, genetic factors may be implicated in the genetic etiology of IGHD in our cohort.

Sources of Research Support: FAPESP grants 05/04726-0 and 07/56490-5; CNPq grants 301477/2009-4, 301339/2008-9 and 300982/2009-7.

Nothing to Disclose: MMF, AALJ, KSA, LRC, BBM, LA, AC, MTD, IJPA

Pub # P2-745
Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Pediatric Endocrinology: Turner Syndrome, Growth, Pituitary, Congenital Adrenal Hyperplasia & Adrenal (1:30 PM-3:30 PM)
Title Is the Glucagon Stimulation Test Useful in the Diagnosis of Childhood Growth Hormone Deficiency?
Author String TE Pinto, AS Mokashi, EA Cummings
IWK Health Centre, Halifax, Canada

Body The efficacy of various pharmacological stimuli used to diagnose growth hormone deficiency (GHD) remains suboptimal. The insulin tolerance test, though the gold standard, is not typically used in young children due to significant side effects. Glucagon is often used in growth hormone stimulation testing in children because of its less severe side effect profile. However, a recent study by Secco et al. reported falsely normal GH response to glucagon stimulation in young children with known risk factors and abnormal response to arginine and insulin. This raises concerns about the clinical utility of the glucagon stimulation test for GHD. We aimed to determine the rate of discordance between glucagon and arginine stimulation tests among patients tested for GHD. A retrospective chart review was performed on all children who had a glucagon stimulation test between 1998 and 2010 at our tertiary care centre. Twelve patients aged 1.5-13 years underwent glucagon stimulation testing. All also had arginine testing. Reasons for GH testing included: small anterior pituitary, hypoplastic pituitary stalk and ectopic posterior pituitary (3); intrauterine growth restriction (IUGR) and birth asphyxia with short stature(1); history of cranial radiation (1); congenital heart defect and poor growth (1); hypoglycemia (2); Prader-Willi syndrome with normal BMI (3) and isolated low growth velocity (1). Ten of 12 (83%) showed an abnormal GH response to both arginine and glucagon (peak <8ug/L). One had a normal response to both tests. Only one had a normal peak GH level with glucagon but not with arginine. This patient had a myelomeningocele and Chiari malformation and recurrent hypoglycemia but not poor growth. All 10 patients testing GHD received GH. One patient with IUGR who discontinued GH due to a decline in response had a normal GH response to clonidine 7 years after initial testing. This chart review demonstrates that glucagon stimulation testing gives comparable results to arginine when used in pediatric patients. Only one patient had a discordant result between arginine and glucagon testing but was not being investigated for poor growth. One other patient did not respond optimally to therapy despite 2 abnormal stimulation tests and then went on to have a normal clonidine stimulation test, either reflecting the overall inadequacies of stimulation testing or suggesting a transient GHD. Our results support the clinical utility of the glucagon stimulation test for GHD.

Secco A et al., J Clin Endocrinol Metab 2010; 95(5):2132

Disclosures: TEP: Coinvestigator, Eli Lilly & Company. ASM: Coinvestigator, Eli Lilly & Company. EAC: Principal Investigator, Eli Lilly & Company.

Pub # P2-746

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Pediatric Endocrinology: Turner Syndrome, Growth, Pituitary, Congenital Adrenal Hyperplasia & Adrenal (1:30 PM-3:30 PM)

Title Serum IGF-I Significantly Correlates with Height SDS in Healthy Young Children

Author String RE Horikawa, N Arata, Y Ohya
National Center for Child Health and Development, Tokyo, Japan; National Center for Child Health and Development, Tokyo, Japan; National Center for Child Health and Development, Tokyo, Japan

Body

Background
Serum IGF-I levels are determined by GH secretion and nutritional status in children. Circulating IGF-I is mainly produced from liver. Liver IGF-I has been reported to have less effect on longitudinal body growth; it has an important effect on metabolism, while locally produced IGF-I such as at bone tissue has growth-promoting effect by paracrine or autocrine fashion.

Objective
To study the effect of serum IGF-I and other factors on body growth in healthy young children.

Subjects and methods
562 children are involved in this study. These children are participants of birth cohort study at NCCHD. The auxological data were obtained at birth, 3,5,6 years of age, and biochemical data were obtained by blood sampling at 5 and 6 years of age. Maternal auxological data and past history were obtained from the maternal and child health handbook. All the data were collected after obtaining informed consent from each participant. Serum IGF-I and other biochemistry of blood samples from children were measured by commercial assays. Statistical procedure using linear regression analysis and t-test was performed after cleaning data.

Results
Serum IGF-I levels distribute from 42.1 to 250.4 ng/ml at 5 years of age and 78.2 to 280.3 ng/ml at 6 years of age. Height SDS were from -2.5SD to +2.7SD at 5 years and -2.3SD to +2.96SD at 6 years. Serum IGF-I and height SDS showed significant positive correlation at both 5 years and 6 years ($P < 0.01$). Height SDS had significant positive correlation with maternal height SDS at 5 and 6 years ($P < 0.01$). Serum IGF-I showed positive relationship with body weight, however, not statistically significant. Birth weight nor length showed no significant correlation with IGF-I at 5 or 6 years of age. Other biochemical factors such as insulin, total protein, cholesterol (total and HDL) had no correlation with body size or IGF-I levels.

Discussion
Serum IGF-I correlates individual height, suggesting that circulating IGF-I has effects on body growth in healthy normal children.

Nothing to Disclose: REH, NA, YO

Pub # P2-747

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Pediatric Endocrinology: Turner Syndrome, Growth, Pituitary, Congenital Adrenal Hyperplasia & Adrenal (1:30 PM-3:30 PM)

Title Hematuria after Arginine Infusion during a Growth Hormone (GH) Stimulation Test: A Case Report

Author String P Armada, S Yigit, M Rasoulpour, K Sardegna, P Phulwani
Connecticut's Children Medical Center, Hartford, CT; Connecticut's Children Medical Center, Hartford, CT

Body Objective: to present an unusual case of hematuria following the administration of Arginine during a GH stimulation test.
Case Presentation: An 8.5 year old male was initially evaluated for short stature (height and weight at the 10th percentile) and no other physical findings. The initial work-up was normal, including bone age, CBC, IGF-1, IGFBP-3, creatinine, and a celiac screen. He was reevaluated for growth deceleration at the age of 15 years. Height was at the 2nd percentile and bone age was delayed at 12 years 6 months. Arginine stimulation test was performed to assess growth hormone production. Thirty hours after the test, the patient developed spontaneous gross hematuria, with no associated dysuria, back pain or frequency. Examination of the urine revealed numerous isomorphic RBC's, with no casts, WBC's, or bacteria present. Urine culture was negative. Abdominal CT scan revealed mild pelvicaliceal dilation of the left kidney with a normal right kidney. A retrograde pyelogram/cystoscopy demonstrated blood and clots from the proximal ureteral orifices; no stones or lesions were identified. Patient received intravenous hydration. Serial urinalysis showed persistent hematuria, which improved after 4 days. Evaluation for autoimmune disorders, vasculitis, glomerulonephritis and bleeding disorders were negative. The patient maintained normal renal function and urine output. Results of the Arginine stimulation test revealed a GH peak of 9.3 ng/dl at 90 minutes. One month later, patient still had hematuria, but this is improving.
Discussion: The Arginine stimulation test is commonly used to assess GH levels. There are only 6 other cases reported in the literature of hematuria following Arginine. Hematuria can present even 2-3 days after Arginine administration. The mechanism is unclear. Osmotic nephropathy was considered because the infusion is hypertonic, but it was excluded because there were no casts consistent with glomerular bleeding, and no changes in renal function.
Conclusion: Intravenous Arginine infusion is widely used for GH stimulation tests and is usually safe. This case illustrates the need to raise awareness of hematuria as a potential adverse reaction. More research is needed to identify the pathophysiology and treatment approach for Arginine induced hematuria.

Nothing to Disclose: PA, SY, MR, KS, PP

Pub # P2-748

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Pediatric Endocrinology: Turner Syndrome, Growth, Pituitary, Congenital Adrenal Hyperplasia & Adrenal (1:30 PM-3:30 PM)

Title Postnatal Growth and Growth Factors in Diseases of Prematurity: Implications for Affected Angiogenesis in Lung and Retinal Development

Author String CA Lofqvist, A Niklasson, I Hansen-Pupp, E Engstrom, LEH Smith, A-L Hard, D Ley, A Hellstrom
University of Gothenburg, Gothenburg, Sweden; University of Gothenburg, Gothenburg, Sweden; Lund University, Lund, Sweden; Harvard Medical School, Children's Hospital, Boston, MA

Body Attaining appropriate growth in very preterm infants continues to be a challenge during the postnatal period. Preterm birth is followed by a decrease in circulatory levels of IGF-I a protein with important growth-promoting and angiogenic properties.

We investigated the relationship between poor postnatal growth, the IGF system and the development of preterm morbidities i.e. bronchopulmonary dysplasia [BPD] and retinopathy of prematurity [ROP]. A retrospective study was performed in 108 very preterm infants [mean (SD) gestational age (GA) 27 (2.2) wks] with weekly weight measurements and blood samples from birth to a postmenstrual age [PMA] of 36 weeks. Infants who developed preterm morbidity had significantly lower GA and BW but not birth weight (BW) SDS compared to infants with no morbidity. The mean (SD) weight SDS at PMA 36 weeks were significantly lower in infants with preterm morbidity compared to infants with no morbidity, -2.1 (1.1) SDS and -1.6 (0.9) ug/L, respectively, p=0.042.

The number of infants with an weight SD score below -2 SDS increased from birth to PMA 36 weeks in infants with morbidity, from 18 to 31 infants (28% to 48%) and in infants with no morbidity from 8 to 13 infants (18% to 30%), respectively.

All growth factors was analyzed longitudinally both with mean (SD) and median (min-max). No significant differences between infants with preterm morbidity compared to infants with no morbidity was found for IGF II or IGFBP-1 and IGFBP-3 or ALS levels.

Serum IGF-I at PMA 30-33 weeks were significantly lower in infants with preterm morbidity compared to infants with no morbidity, 23 (0.9) ug/L and 32 (1.5) ug/L, respectively, p=0.000 also after adjusting for GA at birth p=0.001. The mean IGF-I:IGFBP-3 ratio at PMA 30-33 was significantly lower in infants with preterm morbidity 2.6% compared to 3.3% for infants with no morbidity, respectively p=0.000 also after adjusting for GA at birth p=0.002.

The best fitted binomial multiple regression model for development of preterm morbidity associated with poor postnatal growth included GA, male gender, BWSDS, and IGF-1:IGFBP-3 ratio at postmenstrual age at 30-33 weeks (r²=0.731, p=0.000). This regression model correctly identified 84% of the infants with no morbidity and 91% of the infants with preterm morbidity.

In minimizing the adverse consequences of early preterm birth, in-depth understandings of the mechanisms involved in the regulation of postnatal growth in very preterm infants seem essential.

Nothing to Disclose: CAL, AN, IH-P, EE, LEHS, A-LH, DL, AH

Pub # P2-749

Session Information POSTER SESSION: CLINICAL - Worldwide Efforts To Improve Endocrine Care (1:30 PM-3:30 PM)

Title Inpatient Glycemic Control: An Update from 576 U.S. Hospitals

Author String C Swanson, CB Cook, DJ Potter, GL Kongable, D McMahon
Mayo Clinic Arizona, Scottsdale, AZ; Mayo Clinic Arizona, Scottsdale, AZ; The Epsilon Group, Charlottesville, VA; Luminous Medical Inc, Carlsbad, CA

Body Many quality improvement organizations are interested in inpatient hyperglycemia, but an assessment of glucose control from a large cross-section of hospitals is lacking. We report an analysis of inpatient glucose data from the largest number of U.S. hospitals (N=576) to date. The Remote Automated Laboratory System-Plus (RALS-Plus) was used to extract point-of-care bedside glucose (POC-BG) tests from participating hospitals from January to December 2009. Patient day weighted mean POC-BG values and hypo/hyperglycemia rates were calculated for patients in the intensive care unit (ICU) and non-ICU. The relationships of POC-BG levels with hospital characteristics were determined. Of the 576 hospitals, 47% were <200 beds, 22% were 200-299 beds, 14% were 300-399 beds and 17% were [ge]400 beds; 533 hospitals had ICUs. Most (71%) were urban community hospitals, 26% rural community, and 3% academic facilities; 48% were located in the South, 20% in the Midwest, 19% in the West and 13% in the Northeast. The study sample differed from the national population of hospitals by size, type and region (p<.05), but was comparable to hospitals who use the RALS-Plus system. A total of 49,191,313 POC-BG measurements were analyzed from 3,484,795 patients. The overall patient-day-weighted mean POC-BG was 167 mg/dL for ICU measurements and 166 mg/dL for the non-ICU. Hyperglycemia (>180 mg/dL) prevalence was 32% for ICU and 32% for non-ICU. Hypoglycemia (<70 mg/dL) prevalence was 6% for ICU and 6% for non-ICU patients. Compared to analyses of 2007 data (1), hypoglycemia in the ICU was less in this data (prevalence of 10% previously), with a shift towards higher glucose levels observed in the ICU in this newer data. For ICU and non-ICU there was a significant relationship between patient day weighted mean POC-BG levels with hospital characteristics, with larger ([ge]400 beds), academic, and Western hospitals having the lowest mean POC-BG (P<.001). Hypoglycemia rates in the ICU and non-ICU were higher among the larger and academic hospitals (P<0.001) and lowest among hospitals in the Northeast. This analysis confirms previous results (1) that hyperglycemia is common in U.S. hospitals, and that variation in glucose control according to hospital characteristics exists in the data. Increased hospital participation in data warehousing is needed to support national benchmarking efforts for development of inpatient hyperglycemia management best practices.

Cook CB, Kongable GL, Potter DJ, Abad VJ, Leija DE, Anderson M. Inpatient glucose control: a glycemic survey of 126 U.S. hospitals. *Journal of Hospital Medicine* 2009 4: E7-E14.

Disclosures: CBC: Principal Investigator, Epsilon Group. DJP: Collaborator, Epsilon Group. GLK: Coinvestigator, Epsilon Group. DM: Collaborator, Luminous Medical. Inc. Nothing to Disclose: CS

Pub # P2-750

Session Information POSTER SESSION: CLINICAL - Worldwide Efforts To Improve Endocrine Care (1:30 PM-3:30 PM)

Title Impact of Inpatient Diabetes Management and Education on Glycemic Control after Hospital Discharge: A Randomized Controlled Pilot Trial

Author String DJ Wexler, C Beauharnais, S Regan, DM Nathan, E Cagliero, M Larkin
Massachusetts General Hospital, Boston, MA; Massachusetts General Hospital, Boston, MA

Body Background: Hospitalization provides an opportunity to overcome barriers to treating high-risk patients with poorly controlled diabetes. The effect of inpatient diabetes management and education on long-term glucose control after discharge is not known. We hypothesized that focused inpatient diabetes management and education would improve glycemic control 6 months after discharge in patients with type 2 diabetes and hemoglobin A1c (A1C) $>7.5\%$.

Methods: Thirty-one subjects admitted to general medical and surgical wards for reasons other than uncontrolled diabetes were randomly assigned to inpatient diabetes management with medication titration by an endocrinologist and education by a nurse diabetes educator (IDME) or to usual care (UC). Patients with abnormalities of hemoglobin kinetics and those at high risk of hypoglycemia were excluded. We measured glycemic outcomes 6 months after discharge. We report preliminary findings using Fisher exact test to compare categorical outcomes and t-test or Wilcoxon rank-sum test to compare continuous outcomes. (Clinicaltrials.gov registration number: NCT00869362.)

Results: Patients were 55 ± 12.6 years, with median duration of diabetes of 10 (IQR 7-15) years, mean BMI of 34.5 ± 9.2 kg/m² and baseline A1C of $9.68 \pm 1.64\%$; fifty-five percent were on insulin prior to admission, and 68% had cardiovascular disease, with no differences between groups. The rate of follow-up at 6 months was higher in the IDME group (13 of 15) than the UC group (9 of 16; 87 vs. 56%, $p=0.1$). Six months after discharge, there was a trend toward improved glycemia in the IDME compared to the UC group (mean A1C change -1.28 ± 2.33 vs. -0.45 ± 1.08 , $p=0.3$). Six of 13 (46%) IDME patients and 1 of 9 (11%) UC patients had A1C decline greater than 2% at 6 months ($p=0.2$). Among patients not taking insulin at hospital admission, the A1C change at 6 months was -2.57 ± 1.87 .

Conclusion: This is the first randomized trial to study the impact of an IDME intervention on post-discharge glycemic control. The pilot demonstrated a trend toward improved glycemic control among high-risk patients with poorly controlled diabetes six months after hospital discharge. A sample size of 70-120 patients would be required to demonstrate these findings conclusively. If replicated in a larger trial, these findings would suggest that fewer than 3 patients would need to be treated with this intervention in order to reduce A1C by 2% in one patient.

Sources of Research Support: Funded by a Career Development Award from the National Institute of Diabetes and Digestive and Kidney Diseases to DJW (K23 DK 080 228).

Nothing to Disclose: DJW, CB, SR, DMN, EC, ML

Pub # P2-751

Session Information POSTER SESSION: CLINICAL - Worldwide Efforts To Improve Endocrine Care (1:30 PM-3:30 PM)

Title Long-Term Effectiveness of a Diabetes Self-Management Education Program: 10-Year Data from the Caritas Diabetes Care Centers

Author String E Spanakis, J Paulus, P Mehta, P Andre, JL Griffith, A Sweeney
Caritas St Elizabeth's Medical Center, Boston, MA; Tufts University School of Medicine, Boston, MA; Metro West Medical Center, Framingham, MA

Body

Objective: Diabetes self-management education programs (DMSE) have been shown to be effective in improving glycemic control in at least the short-term period (<1 year of education), though data regarding the long term effectiveness are limited. Our aim was to evaluate the long term impact of our DSME program on clinical outcome measures, hemoglobin A1C (HbA1C), total cholesterol (TC), HDL, LDL and triglycerides (TG).

Methods: A retrospective multicenter study was performed analyzing the records of all patients who attended our Caritas diabetes education centers between years 2000-2010. We analyzed the following outcome measures: HbA1C, TC, HDL, LDL and TG. Patients were included in each analysis if they had at least one additional laboratory visit following their baseline visit in the program. Repeated measures random intercept models adjusting for the baseline value of each lab value were used to evaluate longitudinal change.

Results: 1248 patients were available for the primary analysis of HbA1C with a mean age of 61 years (SD=13). The median duration of follow-up was 293 days (Inter-quartile range: 157 days-857 days). At their first visit, patients had the following mean values: HbA1C 7.94 (SD=2.0), total cholesterol 176.7 (SD=48.5), LDL 97.0 (SD=36.6), HDL 42.8 (SD=13.9) and TG 80.1 (SD=60.8). Compared to baseline visit, the mean HbA1C was significantly decreased in years 1 (0.91 points reduction, $p<0.001$), 2 (0.5 points reduction, $p<0.001$) and 3 (0.24 points reduction, $p<0.001$) but not in years 4 ($p=0.504$) and 5 ($p=0.11$). TC was decreased by 6.9, 2.3, 8.1, 10.4 and 8.4 points in year 1, 2, 3, 4, and 5 respectively. Decreases were statistically significant ($p<0.01$) for all years except year 2 ($p=0.41$). LDL values were significantly decreased in years 1 (4.1 point reduction, $p=0.001$), 4 (6 point reduction, $p=0.006$) and 5 (4.7 point reduction, $p=0.04$) with the exception of years 2 ($p=0.13$) and 3 ($p=0.13$). TG levels were decreased only in year 1 (9.9 points, $p=0.004$), where in years 2,3,4 and 5 no significant difference was observed ($p>0.05$). HDL values remained stable until significant decreases were observed in year 4 (1.8 point reduction, $p=0.02$) and year 5 (1.7 point reduction, $p=0.04$).

Conclusion: DMSE was effective in reducing HbA1C as long as 3 years from the initiation of the program. An improvement in TC, LDL and TG was also observed in most of the follow-up years, though an unfavorable effect on HDL was observed in years 4 and 5.

Nothing to Disclose: ES, JP, PM, PA, JLG, AS

Pub # P2-752

Session Information POSTER SESSION: CLINICAL - Worldwide Efforts To Improve Endocrine Care (1:30 PM-3:30 PM)

Title Effect of Adult Day Health Care Program on Glycemic Control in the Elderly Diabetic Population

Author String LM Loney-Hutchinson, S Kaner, W Edwards, S Schindler, L Nixon
Dr Susan Smith McKinney Nursing and Rehabilitation Center, Brooklyn, NY

Body

Introduction: Diabetes now affects at least 20% of the population over the age of 65(1). Innovative ways of addressing and treating diabetes in this population are needed especially with the advancing number of elderly people in our population. Our study sought to improve diabetes care in the elderly by implementing diabetes interventions in local community Adult Day Health Care programs. The goal of our study was to improve the diabetes care of the Adult Day Health Care registrants with Type 2 Diabetes Mellitus at Dr. Susan Smith Mckinney Nursinig and Rehabilitation Center (DSSM). Our site was part of a larger study conducted by the Adult Day Health Care Counsel which included other Adult Day Health Care Centers in New York State. Our endpoint was improvement in HgBA1c to a goal of <7.5 in this elderly diabetic population.

Method: At the beginning of the study, 35 DSSM Adult Day Program Participants with Type 2 DM signed a consent and HIPAA authorization form. The primary care physician for each patient was contacted by phone and/or letter. Baseline HgBA1c levels were obtained by fingerstick with a point of care HgBA1c machine at the Adult Day Program. Subsequent HgBA1c fingersticks were obtained every three months for each patient for the next year according to the usual standard of care using the same machine. Barriers to treatment goals and co-morbid factors were assessed. Interventions included an interdisiplinary conference with each registrant. The care plan addressed dietary, nursing, social work, medication and therapeutic recreation needs. Contact with the primary care physician regarding possible medication adjustments were also made.

Results: At baseline there were 8 registrants with a HgBA1c >7.5. After implementation of the interventions there were only 4 registrants with a HgBA1c>7.5. The most important barriers to treatment were cultural differences, beliefs and compliance.

Discussion: Our study showed that diabetes management could be improved by using the Adult Day Health Care Center as a source to evaluate diabetes and by implementing focused intensive patient education. Our study is consistent with previous studies which showed that similiar diabetes education interventions in the elderly are effective in improving knowledge and skills to deal with treatment requirements and improving HgBA1c levels significantly(2). Further studies are needed in a randomized control fashion to ascertain our results.

1. Meneilly, D. Tessier, Diabetes in the Elderly. Diabetic Medicine 12(11). pp 949-960,1995
2. Garcia Rosario, Suarez Rolando, Diabetes education in the elderly: a 5-year follow-up of an interactive approach. Patient Education and Counseling 29(1). pp 87-97, 1996

Nothing to Disclose: LML-H, SK, WE, SS, LN

Pub # P2-753

Session Information POSTER SESSION: CLINICAL - Worldwide Efforts To Improve Endocrine Care (1:30 PM-3:30 PM)

Title Diabetes Management in the Community-Dwelling Medicare Beneficiary Population

Author String EA Koller, GS Adler
Centers for Medicare and Medicaid Services, Baltimore, MD; Centers for Medicare and Medicaid Services, Baltimore, MD

Body **Background:** Epidemiologic knowledge about diabetes (DM) and its management, particularly in the elderly and disabled, is limited. **Methods:** De-identified, cross-sectional data were extracted from the 2004 Medicare Current Beneficiary Survey (MCBS) in which 15559 beneficiaries, residing either in the community or long-term care facilities, or their caregivers were queried via interviews. General questions provided data about demographic traits, functional status, and disease burden. Supplemental questions provided data about DM. Descriptive and analytic statistics were used to characterize the population. **Results:** 14500 community dwelling beneficiaries were identified (≥ 65 yrs: 11015, eligible by disability and ≥ 65 yrs: 1003, and eligible by disability and < 65 yrs: 2482. Prevalence of DM and pre/borderline diabetes was 16% and 3%, 28% and 4%, and 18% and 3% in these 3 populations. In those with DM and pre/borderline diabetes, the prevalence of coronary heart disease (CHD) and stroke (CVA) was 38% and 11%, 52% and 19%, and 37% and 11% respectively. In the non-disabled, aged cohort with DM, 73% reported receiving a foot exam in the prior year, 72% an eye exam in the prior year, 78% the influenza vaccine (or nasal spray) in the prior year, 73% the pneumococcal vaccination, 76% HbA1c testing in the last 6 months, and 34% DM self-management training. Cholesterol testing within the last 6 months was highest in the non-disabled, aged cohort with both DM and CHD, 83%, and in the cohort with DM without CHD, 78%, versus 71% in the CHD without DM cohort. Similarly, blood pressure testing within the last 6 months, was highest those with DM whether with/without CHD, 96% and 94% (versus 94% in those with prior CVA and 81% in those with CHD. Smoking rates were lowest in the non-disabled, aged cohort with DM and CHD, 7%, and highest in the young disabled cohort with pre/borderline diabetes and with or without CHD, ~40%. The young, disabled cohort often received fewer medical assessments. **Conclusion:** The burden of DM and CHD is significant in all of the Medicare populations. Patients with DM receive more cardiovascular assessments than patients with CHD without DM. Further analysis will attempt to identify patient characteristics that contribute to better patient management and health behaviors. Variables such as marital status, secondary insurance availability, functional status, underlying disease process, and co-morbid disease burden will be explored.

Sources of Research Support: No outside support. These findings do not necessarily represent the positions of the Federal government or the Center for Medicare and Medicaid Services.

Nothing to Disclose: EAK, GSA

Pub # P2-754

Session Information POSTER SESSION: CLINICAL - Worldwide Efforts To Improve Endocrine Care (1:30 PM-3:30 PM)

Title Effectiveness of Online Training in Improving Inpatient Diabetes Management by Internal Medicine Residents

Author String A Wong, SP Ramirez Rodriguez, NI Steinle, L Reinhart
University of Maryland, Baltimore, MD

Body In the United States, 12 to 25% of patients admitted to the hospital regardless of their diagnosis at admission have diabetes. From 1980 to 2003, the diagnosis of diabetes at admission increased 2.3 fold. Hyperglycemia is associated with adverse outcomes, increasing length of stay and overall cost of healthcare. The overall aim of this project was to evaluate the effectiveness of an online training program in improving Internal Medicine residents' management of diabetes among hospitalized patients. A non-randomized, retrospective, prospective study was performed. All charts with admission ICD-9 codes of diabetes that were cared for by internal medicine residents at a major academic center in August 2009 were reviewed. An online training course of 10 modules was made available to the residents in November 2009. A subsequent chart review was performed of patients admitted in December 2009 and cared for by residents who completed all modules to assess for diabetes management. Use of basal and prandial insulin, incident hypoglycemia and frequency of request for consultation from the diabetes service were evaluated. A total of 98 charts were reviewed for each of the pre and post training periods. Of the 137 residents who were eligible to complete the online diabetes training course, 105 (76%) completed all 10 modules. Of the remaining 32 residents, 13 (10%) completed some of the modules while 19 (14%) did not participate. We observed a trend toward increased use of basal insulin in the post (68.7%) vs. the pre (52.5%) education period. This trend did not reach statistical significance ($p = 0.45$). If the observed trend is stable, a sample size of approximately 140 subjects in each group is needed in order to have 80% power to detect a significant difference between the two groups at an alpha level of 0.05. We also observed a small increase in hypoglycemia events in the post (14/98) training period vs. pre (9/98), which was not statistically significant ($p = 0.20$). We would need a sample size of 630 in each group in order to have 80% power to detect a significant difference. Requests for diabetes consultation services were 2% in both the pre and post training periods. Online training may be an effective method of training medicine residents regarding the treatment of hospitalized patients with diabetes. Although our study showed trend towards greater use of insulin, it did not reach statistical significance, probably due to the limited sample size.

Nothing to Disclose: AW, SPRR, NIS, LR

Pub # P2-755

Session Information POSTER SESSION: CLINICAL - Worldwide Efforts To Improve Endocrine Care (1:30 PM-3:30 PM)

Title Sustainability of Resident Education in Follow-up of Newly Diagnosed Hyperglycemia

Author String LP Lonappan, S Srivastav, RA Fitzpatrick
Crozer Chester Medical Center, Upland, PA

Body

Aim: To evaluate sustainability of targeted resident education in follow up of newly diagnosed hyperglycemia in hospitalized patients

Background: Neglect to follow up hyperglycemia in hospitalized patients is a missed opportunity for early diagnosis and management of diabetes. In 2009 a study by the same authors showed that resident education over a period of one month improved follow up of newly diagnosed hyperglycemia, in terms of ordering FPG in patients with high glucose on admission (100%), ordering A1C (33%), insulin orders(23%) and discharge instructions to follow up with PCP (46%) .With no further targeted resident education over the next 12 months, we did a similar study to assess sustainability of the past education.

Method: Patients [ge] 21 yrs without history of diabetes and with BG >100, admitted to teaching services between 11/01/2010 and 12/01/2010 were included. Patients with DKA and ICU admissions were excluded. Retrospective chart review was done regarding resident orders for FPG, A1C, diabetes teaching and discharge instructions for PCP follow up. We compared our results with the 2009 study. We also conducted a survey where the residents were asked at what level of BGs they would follow up inpatient hyperglycemia and their perceived barriers in doing so.

Results: Of the 205 charts reviewed, 150 met inclusion criteria. FPG were ordered in all cases. A1C was ordered in only 34.6% cases (52/150), of which 27/52 was found to be [ge] 5.8. There were 21 pre-diabetics (A1C 5.8-6.4) and 6 diabetics (A1C [ge]6.4). Diabetes education and discharge instructions were ordered in 4/27(< 5%). In the survey, completed by 18 residents, 70 % said they would check A1C and 72% said they would order outpatient follow up when FPG is 125-200. The major barrier in follow up was identified as coexisting acute issues in inpatients that take priority over management of hyperglycemia.

Conclusions: There was only modest sustainability with our intensive targeted educational program a year ago. Residents fared poorest in terms of discharge instructions for newly diagnosed hyperglycemia. There is room for much improvement. Although the resident survey suggested adequate knowledge, our chart review revealed that this was not seen in practice. We recommend that measures should be taken to address this knowledge and practice gap. Measures may include intermittent reinforcement via lectures and emails, computer based prompts, and personal feedback to residents.

Nothing to Disclose: LPL, SS, RAF

Pub # P2-756

Session Information POSTER SESSION: CLINICAL - Worldwide Efforts To Improve Endocrine Care (1:30 PM-3:30 PM)

Title Increased Incidence of Extreme Hyper- and Hypoglycemia in Hospitalized Patients during the Month of July in Teaching Hospitals

Author String M Cheikh, S Saluja, M Shomali
Union Memorial Hospital, Baltimore, MD

Body

Introduction
Blood glucose (BG) control has been found to be an important component to the care of hospitalized patients. Maintaining BG within a target range using intensive insulin is challenging and requires skill and experience. We hypothesized that there may be an opportunity for improved outcomes in the month of July, when new resident physicians begin their training.

Methods
We reviewed data of point-of-care BG's of hospitalized patients on different medical and surgical care units at 2 community teaching hospitals. We defined serious hypoglycemia as a BG < 41 and severe hyperglycemia as a BG >399.

Results
We found 2 care units which had significantly more hyperglycemia in July than other months: (1) 60 cases in July, monthly mean 23 +/- 15 cases; (2) 36 cases in July; monthly mean 13+/-10 cases. We found 1 care unit with significantly more hypoglycemia in July: 13 cases; monthly mean 6+/-3 cases. All three of these units admitted patients with coverage by resident physicians. In units with patients cared for exclusively by physician assistants and nurse practitioners, no increase in the extremes of BG's were identified in July.

Conclusions
These data suggest that there is an increased risk of extreme hyper- and hypoglycemia during the month of July at teaching hospitals. Diabetes management protocols and training programs for new resident physicians may need to be implemented immediately at the beginning of training.

Nothing to Disclose: MC, SS, MS

Pub # P2-757

Session Information POSTER SESSION: CLINICAL - Worldwide Efforts To Improve Endocrine Care (1:30 PM-3:30 PM)

Title Random Plasma Glucose Predicts Diagnosis of Diabetes in Veterans in the Southeastern United States

Author String DE Olson, M Zhu, Q Long, CL Jasien, JL Byrd-Sellers, LS Phillips
Atlanta VA Medical Center, Decatur, GA; Emory University School of Medicine, Atlanta, GA; Rollins School of Public Health, Emory University, Atlanta, GA; Atlanta VA Medical Center, Decatur, GA

Body

Background: Early intervention can prevent or delay development and progression of diabetes and its complications, but is precluded if patients at risk are unrecognized. Since veterans receiving care through the Veterans Health Administration are not screened for diabetes in any systematic way, we asked whether outpatient random plasma glucose (RPG), frequently included in veterans' blood tests, could predict short-term risk of unrecognized diabetes.

Methods: Using the Veterans Integrated Service Network (VISN) 7 Corporate Data Warehouse (data from South Carolina, Georgia, and Alabama), patients without previously diagnosed diabetes were included if they had outpatient RPG measurements and consistent primary care follow-up (at least 9 visits over at least 7 years). Diagnosis was determined by use of the diabetes ICD-9 code (250.xx) at a primary care visit or meeting VA Diabetes Epidemiology Cohort criteria (any use twice, or prescription of a diabetes drug). Those diagnosed with diabetes were compared to controls without a diagnosis. The ability for RPG values to predict a diagnosis of diabetes within the subsequent several years was assessed by Receiver Operating Characteristic (ROC) analysis.

Results: 15,794 subjects met follow-up criteria and had >1 RPG values in 1999-2001, and diabetic and control subjects were comparable in age, gender, and race (all p=ns). The peak, average, and second highest RPG were similar in predicting a diagnosis of diabetes within the next 3 years (ROCs 0.75, 0.76, and 0.76, respectively). Two RPG values of >120 mg/dl in 1999-2001 provided sensitivity of 57% and specificity of 79% for diagnosis of diabetes within the next 3 years, while two values of >150 mg/dl provided sensitivity of 21% and specificity of 95%. The accuracy of prediction of a diagnosis of diabetes within the next 3 years increased progressively from two RPGs in 1999-2000 (ROC 0.72) to two RPGs in 2002-2003 (ROC 0.82), p<0.001.

Conclusions: RPG obtained during outpatient visits predicts the diagnosis of diabetes within the following three years. RPG levels below the diabetes [ldquo]diagnostic[rdquo] range provide good specificity and sensitivity to predict a subsequent diagnosis. More recent measurements are stronger predictors.

Impact: Use of RPG to signal the need for further diagnostic testing might lead to earlier identification of veterans with undiagnosed diabetes and prediabetes, who could benefit from preventive management.

Sources of Research Support: VA HSR&D SHP 08-144 and IIR 07-13.

Nothing to Disclose: DEO, MZ, QL, CLJ, JLB-S, LSP

Pub # P2-758

Session Information POSTER SESSION: CLINICAL - Worldwide Efforts To Improve Endocrine Care (1:30 PM-3:30 PM)

Title Relationship between Adherence of Physicians to SEMT Diabetes Guidelines and the Degree of Glycemic Control in Type 2 DM Patients in Turkey

Author String I Satman, S Imamoglu, C Yilmaz, ADMIRE Study
Istanbul University, Istanbul Faculty of Medicine, Istanbul, Turkey; Uludag University Faculty of Medicine, Bursa, Turkey; Ege University Faculty of Medicine, Izmir, Turkey

Body **Background:** Clinical practice guidelines on diabetes mellitus (DM) have been revised in 2009 by The Society of Endocrinology&Metabolism, Turkey (SEMT). Ongoing ADMIRE Project is designed to evaluate the effect of implementation activities to increase physicians' awareness on guidelines. Results of prospective phase are presented.
Methods: 180 physicians kept 6 months medical records of their type 2 DM patients, without any interference to their routine practice. Scores of degree of adherence were calculated. This report depends on analysis of data of 885 patients included in prospective phase.
Results: About 2/3 (62%) of patients were women. Mean DM duration and mean age were 7.1±6.9 yrs and 55.3±10.4 yrs, respectively. 46% of patients were advised to take OAD, 11% insulin and 25% OAD+insulin. Overall, medical history (HISTORY) was taken with full adherence to SEMT guidelines in 40.6% of patients. However, only 8.5% of patients had physical examination (PHY_EXAM) done with full adherence. None of the patients had laboratory evaluation (LAB_EVAL) done with full adherence. Scores of adherence to guidelines (over 100%) were 52±15, 68±32, 47±21 and 41±16, corresponding to general adherence and clinical practices on HISTORY, PHY_EXAM and LAB_EVAL. Only 15.3% of patients were at A1C target (≤6.5%) at first visit of prospective phase. This figure increased to 20.5% at follow-up visit. Corresponding figures for patients achieved acceptable glycemic control (A1C ≤7.0%) were 26.8% at first and 38.7% at six-month visits, respectively. Proportion of patients at FBG (70-120 mg/dl) and PPBG target (≤140 mg/dl) increased from 17.5% and 12.6% to 31.3% and 19.9% during 6-months' follow-up, respectively. Mean A1C level decreased from 8.6±2.0% to 8.2±1.7% and 8.1±1.7% in patients with adherence score <50% and 50-75% and >75% (p=0.013). Mean FBG was 190±78 mg/dl in patients with adherence score <50%, but decreased to 169±63 mg/dl and 160±46 mg/dl with adherence score 50-75% and >75%, respectively (p<0.001).
Comment: Overall adherence to SEMT DM guidelines was not so good. Practices of HISTORY taking were in moderate consistence with guidelines, but adherence to guidelines regarding PHY_EXAM and LAB_EVAL was very low. Furthermore, glycemic control is better maintained with increasing overall adherence to guidelines. Therefore, it is suggested that physician training programs should focus, in depth, the areas with lack of knowledge and/or reluctance to adhere with the guidelines.

(I) Satman I, Yilmaz C, Imamoglu S and Diabetes Study Group of SEMT: Clinical Practice Guidelines for Diagnosis, Treatment and Follow-Up of Diabetes Mellitus and Its Complications, 2009.

Nothing to Disclose: IS, SI, CY

Pub # P2-759

Session Information POSTER SESSION: CLINICAL - Worldwide Efforts To Improve Endocrine Care (1:30 PM-3:30 PM)

Title An Interdisciplinary Education Program Training Health Professionals to Encourage Physical Activity in Patients with Type 2 Diabetes

Author String O Giet, D Sofra, T Cancelli, L Allet, P Marques-Vidal, J Ruiz, N Pitteloud, H Delgado, M Castellsague, C Negre, S Beer, JJ Puder
University of Lausanne, Lausanne, Switzerland; University of Geneva, Geneva, Switzerland

Body **Background:** Innovative and feasible ways to encourage physical activity (PA) in diabetic patients are needed. We aimed to evaluate a new interdisciplinary program encouraging health professionals in the community to establish community-based PA programs for patients with type 2 diabetes.
Methods: The one day interdisciplinary education program took place in the French part of Switzerland in November 2010 and included theoretical presentations, therapeutic education skills training, practical PA sessions, interactive discussions with diabetic patients participating in ongoing PA programs and profession-specific workshops. All participants were given a 47-item evaluation form including both closed and open questions.
Results: Seventy-one health professional participated (23 physicians, 18 dieticians, 16 sport therapists and 14 diabetes educators) and all filled out the evaluation form. Ninety-three % or more of the participants stated that such an education program was innovative and found it to be adapted or very adapted to their daily practice. Ninety-three % or more found all aspects of the program both interesting or very interesting and helpful or very helpful for their daily practice with the exception of therapeutic education which was helpful or very helpful for 86% of the participants. Fifty-five % of the participants rated the theoretical presentations, 49% the therapeutic education sessions, 51% the practical PA sessions, 71% the discussion with patients and 43% the profession-specific workshops as being very interesting. They were no gender or health profession-specific differences in the evaluation (all p=NS). In response to the open question [ldquo]what is your general impression of this education program[rdquo], 33/37 responses mentioned the interdisciplinary nature to be an innovative and outstanding strength of the program.
Discussion: This innovative interdisciplinary education program was equally attractive across different health professions working in the community. The perceived outstanding strengths were the interdisciplinary nature and the discussion with patients. Further novel approaches of training programs and their effects on modifying patient behavior will be evaluated in the future.

Nothing to Disclose: OG, DS, TC, LA, PM-V, JR, NP, HD, MC, CN, SB, JJP

Pub # P2-760

Session Information POSTER SESSION: CLINICAL - Worldwide Efforts To Improve Endocrine Care (1:30 PM-3:30 PM)

Title Implementation of a Multidisciplinary Strategy Promoting Basal Bolus Insulin Results in Improved Glycemic Control and Shorter Length of Stay for Diabetic Inpatients

Author String K Helmle, A Dechant, A Edwards
University of Calgary, Calgary, Canada

Body **Objective:** To determine medical residents' practices regarding inpatient diabetes management, insulin use and to implement and evaluate an educational initiative promoting basal bolus insulin therapy (BBIT). We hypothesize that BBIT will reduce the frequency of hyper and hypoglycemia and decrease length of hospital stay when compared to sliding scale insulin (SSI) use.

Methods: Data from a retrospective audit of electronic health records for patients admitted to the Medical Teaching Unit (MTU) over a 12 month period prompted the introduction of a new diabetes management protocol emphasizing BBIT as a replacement for commonly prescribed SSI. A subsequent chart audit over the following 9 months evaluated the proportion of patient-days that met the Canadian Diabetes Association published glycemic targets, the frequency of hypoglycemic events, and the length of hospital stay compared to patients treated with SSI.

Outcomes: 724 patients (37031 glucose results), and 355 patients (18600 glucose results) were analyzed pre- and post-intervention, respectively. Pre-intervention, patients prescribed SSI had an increased frequency of hyperglycemia (BG>18mmol/L (324mg/dl), 4.4% vs. 8.1% p<0.0001), hypoglycemia (BG<4mmol/L (72mg/dl), 3.5% vs. 3.1%, p=0.045), and a longer length of stay (27.7 days vs. 16.0 days, p=0.0002) when compared to those not treated with SSI.

An intensive educational initiative including multi-disciplinary seminars, pocket-cards and a web-based teaching tool focussing on BBIT was promoted on the MTU. 355 diabetic patients, resulting in 18600 blood glucose results were analyzed following the intervention. Frequency of BBIT prescription doubled following the intervention. Initial post-intervention data suggested that BBIT, when compared to SSI, was associated a 37% increase in patient-days entirely within glyceic targets (32.0% vs. 43.8% on BBIT, p<0.001), without a statistically significant increase in the frequency of hypoglycaemia (3.3% vs. 4.4% on BBIT, p=0.06). Length of stay was dramatically shortened for those treated with BBIT (28.2 days vs. 13.2 days, p<0.001).

Conclusions: Our BBIT protocol maintains patients' blood glucose within target more frequently and is associated with reduced length of stay when compared with SSI. We have designed an electronic order set to standardize BBIT prescription, and are undertaking a knowledge translation initiative to promote uptake and utilization of BBIT within the multidisciplinary hospital framework.

Nothing to Disclose: KH, AD, AE

Pub # P2-761

Session Information POSTER SESSION: CLINICAL - Worldwide Efforts To Improve Endocrine Care (1:30 PM-3:30 PM)

Title Statewide, Multi-Payer Supported Medical Home Initiative Improves Primary Care Quality

Author String RA Gabbay, B Ebersole, MH Bailit, EH Wagner
Penn State Hershey Medical Center, Hershey, PA; Pennsylvania Governor's Office, Harrisburg, PA; Bailit Health Purchasing, LLC, Needham, MA; Group Health Cooperative, Seattle, WA

Body **Background:** The Patient Centered Medical Home (PCMH) holds significant promise to improve primary care outcomes for chronic illness such as diabetes with several small pilots reporting success. The Pennsylvania Chronic Care Initiative is one of the largest multi-payer PCMH initiatives in the country, involving over 150 primary care practices (in 7 regions of the state) with 17 participating insurers and more than 750 providers.
The effort is aimed at practice redesign with an initial focus on diabetes then moving to other high-risk patients. Participating practices have been supported in implementing the Chronic Care Model through quarterly regional Breakthrough Series learning collaboratives, practice facilitation, and infrastructure payments by participating payers in 4 of the regions and a smaller state grant program in the other 3 regions.
Methods: Staggered regional rollouts of the initiative occurred across the 7 regions of PA starting in May 2008 with new practices being added until December 2009. Cross-sectional data from monthly practice reports for approximately 50,000 diabetes patients statewide was assessed (baseline in September 2008 and a second measurement period two years later in September 2010, nine months after the launch of the seventh regional rollout).
Results: The Pennsylvania initiative registered impressive improvement across a range of diabetes measures. Data reporting from September 2008 to September 2010 reveals significant increases in the absolute percentage of patients at goal for A1C [+8%], blood pressure [+9%] and LDL cholesterol [+11%]. Practices focused on reducing the highest risk populations; the absolute change in percentage of patients with A1C >9 was -12%; LDL <130, +21%; and BP <140/90, +18%. Adherence to complication screening improved as well: eye exams increased by 16%, foot exams by 32%, and nephropathy screening by 19%. Another big gain was in self-management goal setting: +27%. Leading practice changes included reorganizing towards team-based care, incorporation of self-management support and education, planned visits, and office huddles. Many practices began using registries and examining their data for the first time.
Conclusions: A state-led PCMH initiative can significantly improve diabetes care. State government can play a critical role in spreading PCMH by convening multiple payer and provider groups to develop infrastructure support for improved diabetes care.

Nothing to Disclose: RAG, BE, MHB, EHW

Pub # P2-762

Session Information POSTER SESSION: CLINICAL - Worldwide Efforts To Improve Endocrine Care (1:30 PM-3:30 PM)

Title The Patient-Centered Medical Home (PCMH) Improves Diabetes Care

Author String T Bojadziewski, R Gabbay
Penn State College of Medicine, Hershey, PA

Body The Patient Centered Medical Home (PCMH), a new model for primary care, is being proposed to address the demands of the chronically ill, including diabetes. Its characteristics include comprehensive, team based and patient centered care, a whole person approach, enhanced provider accessibility, self management support, patient empowerment and payment reform. Diabetes is a logical chronic illness to benefit from the PCMH and has been a common focus of many national PCMH efforts given its high cost, strong evidence based guidelines, and recognized need for team based care.

Eleven PCMH demonstrations that report quality of care data for diabetes were identified. The most frequent interventions to transform to a PCMH were care coordination, payment reform, diabetes patient registry implementation, learning collaboratives, and practice coaching.

Overall, there were significant improvements in both diabetes process and outcome measures. Results from large integrated health systems include: 1) Group Health (WA) with a 2 year improvement in its bundled [ldquo]Composite Quality Score[rdquo] (51 to 58.6%) for 9200 patients, lower costs with higher provider satisfaction; 2) Geisinger Health System (PA) with first year improvements (n=20,000) in A1C < 7.0 (32.2 to 34.8%, p<0.001), blood pressure <130/80 (39.7 to 43.9%, p<0.0001) the [ldquo]Diabetic Bundle[rdquo] (2.4 to 6.5%) and documented cost savings; 3) Health Partners (MN) improved in the [ldquo]Diabetes Bundle [rdquo] (4 to 25%) over a 4 year period; 4) Genesys Health System (MI) reported 6 month improvements in glucose self monitoring (50 to 91%), foot self examinations (67 to 96%), annual eye exams (37 to 70%) and those who received formal diabetes education (39 to 67%). Early results of large state led initiatives: Pennsylvania absolute change in A1C > 9 (-12%), systolic BP < 130/80 (+9%), and LDL <130 (+21%). Also saw improvements in setting self management goals (+27%), foot examinations (+32%), eye examinations (+16%) and diabetic nephropathy screening (+19%); Colorado and North Carolina both successfully met NCQA diabetes quality benchmarks. Potential cost savings were evident in the majority of demonstrations due to reductions in ER visits and inpatient hospitalizations. Based on early results of several initiatives, the PCMH holds significant promise to improve diabetes outcome and reduce costs.

Nothing to Disclose: TB, RG

Pub # P2-763

Session Information POSTER SESSION: CLINICAL - Worldwide Efforts To Improve Endocrine Care (1:30 PM-3:30 PM)

Title Investigation of Screening Standards for Thyroid Dysfunction and Celiac Disease in Type 1 Diabetes in the West of Ireland

Author String R Casey, A Eddie, M Bell, B Dineen
University Hospital Galway, Galway, Ireland; University of Aberdeen, Aberdeen, UK

Body Thyroid dysfunction (TD) and Coeliac disease (CD) occur with increased frequency in patients with type 1 diabetes (T1DM). Current American Diabetes Association (ADA) guidelines recommend screening all patients with newly diagnosed T1DM by measuring TSH and thyroid antibodies. If initial testing is normal it is recommended to repeat thyroid function tests (TFT) 1-2 yearly thereafter. Screening for CD with anti-transglutaminase antibodies is recommended at diagnosis. Repeat testing should only be performed if patients become symptomatic. Earlier guidelines had recommended screening at diagnosis, year 1 and 2 post diagnosis and thereafter only if symptomatic.

Aims
To assess the screening practices for TD and CD in patients with T1DM at our diabetes centre compared to the 2010 ADA guidelines.

Methods
We completed a retrospective review of patients diagnosed with T1DM between 2004 and 2010. We collected necessary information from our diabetes database and a computer based laboratory system.

Results
213 subjects with T1DM were included in the study. 62% were male and 38% female. 86.4% had TFT at diagnosis. Over the time period studied, a significantly increasing trend in the percentage of subjects with TFT performed at diagnosis was observed ($r^2=0.86$; $p=0.002$). However, only 14.6% were tested according to ADA recommendations; 35.7% did not have TPO antibodies checked at diagnosis, 30.5% had TFT at diagnosis only and did not have annual screening nor TPO antibodies performed at diagnosis and 19.2% were not screened at all for TD. Only 35.7% patients had CD testing. 18.9% were screened according to protocol. 64.3% patients were not screened at all for CD and 8% were not tested at diagnosis but at some later date. Prior to the change in the screening recommendations in 2010, 5.8% were screened according to protocol; with the advent of the new guidelines screening for CD had improved to 33.3% (OR=8.1; 95% CI=2.8-22.9)

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Nothing to Disclose: RC, AE, MB, BD

Pub # P2-764

Session Information POSTER SESSION: CLINICAL - Worldwide Efforts To Improve Endocrine Care (1:30 PM-3:30 PM)

Title Metabolic Syndrome Awareness in Asian Americans and Internet Survey

Author String J Trang, K Tran, R Lam, G Wu
UCLA, Los Angeles, CA; Tulane University, New Orleans, LA; Santa Clara University, Santa Clara, CA; Stanford University School of Medicine, Stanford, CA

Body Metabolic Syndrome affects Asians disproportionately and is related to increased risk of cardiac mortality, the leading of death in America (1,2). Asian-Americans who comprise 4% of the US population, represent 30-50% of the Californian population. Despite the importance of this syndrome in assessing cardiovascular risk, internet interest peaked in 2007 correlating to press reports of diet soda and metabolic syndrome
Purpose: Use of Internet questionnaire to assess knowledge of metabolic syndrome in the Asian community.
Methods: Internet survey was posted on Topix.com, Crunchyroll.com, Asianfanatics.net, Facebook, using Makesurvey.net. Makesurvey.net is an online survey tool that is free. To ensure maximum views, we used Google banner service to direct web traffic to our posted survey. Questionnaire (QN) was about metabolic syndrome, Body Mass Index, diabetes, frequency of doctor visits.
Results: 101 QN: 34(34%) Males, 67 (67%) Females; avg age 24.9+/-6.2 yrs. Ethnicities: Chinese 48 (47.5 %) ; South Asian 9 (6.9%), Other Asians 7 (6.9%); Non Asian 37 (36.6%). Education level: HS=2 (1.9%); college =87(86.1%); graduate school 12 (11.9%). The responses of "don't know" occurred with these two questions: Asians have a higher incidence of diabetes with low BMI (67.3%); awareness of Asians having higher risk of metabolic syndrome than Caucasians (70.3%). However, 41.2% males vs 22.4% females were aware that Asians were at a higher risk of metabolic syndrome than Caucasians (chi square p=0.04).
Conclusion: In this study, Internet survey responders who were mostly college educated demonstrated lack of awareness of the metabolic syndrome, a leading public health problem.

Palaniappan LP et al. Int J Obes 2010; doi: 10.1038 (Aug 3, 2010)
Isoamaa BO et al. Diabetes 2001; 24:683-689.

Nothing to Disclose: JT, KT, RL, GW

Pub # P2-765

Session Information POSTER SESSION: CLINICAL - Worldwide Efforts To Improve Endocrine Care (1:30 PM-3:30 PM)

Title Should You Follow Practice Guidelines in Endocrinology? A Systematic Assessment of Their Methodologica Quality

Author String I Bancos, T Cheng, L Prokop, V Montori, M Murad
Mayo Clinic, Rochester, MN

Body Clinical practice guidelines (CPGs) have been developed to assist practitioner and patient decisions about appropriate health care for specific clinical circumstances and have an increasingly widespread and important role in clinical decision making as well as the determination of financial formularies and payer coverage policies. These far-reaching consequences render it essential for an evaluation of the factors that contribute to the quality of these guidelines and the subsequent understanding of what makes a high quality guideline. Specifically, there have been no published audits of quality of contemporary endocrine guidelines. We have set out to address this deficit, assessing the quality of endocrine CPGs in the past three years. A systematic review of current literature was conducted to identify all endocrine clinical practice guidelines developed in North America and published between January 1st, 2007 and January 12th, 2010. Two independent reviewers used the Appraisal of Guidelines, Research and Evaluation instrument to evaluate the quality of the guidelines in six domains: scope and purpose, stakeholder involvement, rigor of development, clarity and presentation, applicability, and editorial independence. One hundred CPGs were eligible for inclusion in this study. Endocrine guidelines had high scores in the scope-and-purpose and clarity domains but low scores in the stakeholder-involvement and applicability domains. Only 29% of guidelines had standardized scores of more than 60% for more than 3 domains. Rigor-of-development domain score was significantly higher in guidelines using the GRADE (Grading of Recommendations, Assessment, Development and Evaluation) approach, non-diabetes guidelines, having an explicit reporting of funding, and in published in print versus online publications. The quality of endocrine guidelines published in 2007-2009 is moderate, and seems comparable to that of guidelines in other specialties. Guideline developers can improve guideline quality by (a) using methodologically sound development frameworks, (b) increasing stakeholder involvement, and (c) paying more attention to resource implications of guideline implementation.

Nothing to Disclose: IB, TC, LP, VM, MM

Pub # P2-766
Session Information POSTER SESSION: CLINICAL - Worldwide Efforts To Improve Endocrine Care (1:30 PM-3:30 PM)
Title Perspectives on Multidisciplinary Care in the Treatment of Neuroendocrine Tumors
Author String A Heaney, DC Metz, CW Howden, J Strosberg, PA Pellikka, D Kilmstra, J Choi, JC Yao
David Geffen School of Medicine at the University of California, Los Angeles, CA; Perelman Center for Advanced Medicine, University of Pennsylvania, Philadelphia, PA; Northwestern University Feinberg School of Medicine, Chicago, IL; H Lee Moffitt Cancer Center & Research Institute, Tampa, FL; Mayo Clinic College of Medicine, Rochester, NY; Memorial Sloan-Kettering Cancer Center, New York, NY; MD Anderson Cancer Center, Houston, TX

Body **Background:** Neuroendocrine tumors (NETs) are challenging to identify and difficult to treat. The heterogeneity of NET presentation, potential for multisystem complications, and diversity of symptomatology highlight the need for access to numerous specialties/subspecialties with proven experience in managing this disease. Importantly, treatment guidelines for NETs emphasize collaboration among diverse medical disciplines to optimize patient outcomes (NCCN 2010 v.2.2010, pMS-2).
Methods: Patient needs and key considerations for effective multidisciplinary team management were identified. A conceptual model of the organizational structure of multidisciplinary teams in NET management was developed, and the skills and experience of core team members are described.
Results: A permanently staffed core team is the functional unit of a multidisciplinary care model. The core is composed of at least 3-4 clinicians with extensive NET experience, ultimately responsible for decisions made about a patient. The core team often contains the following clinical expertise: gastroenterology, medical oncology, endocrinology, surgery, pathology, and radiology. However, because the needs of NET patients are unique and can change (eg, early disease versus advanced disease), the composition of a multidisciplinary core team will differ and may need to be tailored to the needs of the patient. Guided by clinical need, the core group enlists other team members (for example, cardiology or anesthesiology) with particular expertise in aspects of NET management. A multidisciplinary organizational structure offers significant advantages to patients and providers, such as earlier diagnosis and staging, multidisciplinary discussion and peer review of treatment plans, with the aim of providing evidence-based treatment modalities in a single center. A potential mechanism for effective team interactions is regularly scheduled meetings attended by the core team, where challenging cases are presented and discussed in detail.
Conclusion: A multidisciplinary approach provides the framework to streamline patient care and improve communication among specialists who treat NETs. In addition, patients can gain access to the full range of evidence-based treatment options. This care model has the potential to enhance patient satisfaction, improve outcomes, and augment quality of life.

Sources of Research Support: Novartis Pharmaceuticals Corporation, East Hanover, NJ.

Disclosures: AH: Consultant, Novartis Pharmaceuticals. DCM: Consultant, Novartis Pharmaceuticals; Astra Zeneca; Takeda; Grant Review Panel, Tercica; Medical Advisory Board Member, Novartis Pharmaceuticals. CWH: Consultant, Takeda; Novartis Pharmaceuticals; Proctor & Gamble; Merck & Co.; Schering Plough; Takeda. JS: Consultant, Novartis Pharmaceuticals. PAP: Consultant, Novartis Pharmaceuticals. DK: Consultant, Novartis Pharmaceuticals. JC: Consultant, Novartis Pharmaceuticals. JCY: Consultant, Novartis Pharmaceuticals; Ipsen; Pfizer, Inc.; Takeda; Research Funding, Novartis Pharmaceuticals; Genentech, Inc.

Pub # P2-767

Session Information POSTER SESSION: CLINICAL - Worldwide Efforts To Improve Endocrine Care (1:30 PM-3:30 PM)

Title Group Education Improves Patient Confidence in Managing Steroid Sick Day Rules

Author String LJ Munday, PL Chong, PS Kar
Queen Alexandra Hospital, Portsmouth, UK

Body

Background
Group education for patients with diabetes is a well validated method of enhancing self-management skills. More recently some endocrine departments have used group education to teach patients how to administer IM hydrocortisone.

Innovation
Patients on steroid replacement, identified by the Endocrine Nurse and General Practitioners, were invited to attend a group session. The aim was to ensure every relevant patient in the locality had the opportunity to be educated about dose adjustment in times of illness etc, and supplied with an emergency hydrocortisone injection.

Method-
Up to eight patients were invited to attend each 1 hour session. If they wished each patient could bring a relative or friend with them. Using group discussion and problem solving exercises patients were educated regarding steroid replacement therapy dose adjustment, and administration of emergency hydrocortisone injection. Patient confidence regarding steroid medication adjustment during intercurrent illness, was measured using a likert scale pre and post group session.

Results
57 patients: 21 Addison's, 30 hypopituitarism, 3 Cushing's, 2 CAH, 1 isolated ACTH deficiency.
Likert scale 1= not confident, 10 = very confident.

Confidence level
Addison's Pre group: 6.19 Post group: 9.86
Hypopituitary Pre group: 4.73 Post group: 8.03
Cushings Pre group: 4.0 Post group: 9.0
CAH Pre group: 5.5 Post group: 10.0
ACTH def. Pre group: 7.0 Post group: 10.0

Conclusion
Patients who have participated in a group education session have higher levels of confidence regarding managing their steroid replacement therapy ($p=0.003$). The increase in confidence is demonstrated irrespective of their diagnosis. In addition patients enjoyed the group sessions and welcomed the opportunity to meet other people with similar medical conditions. The challenge now is to maintain the patients self management skills which may require development of a new group education programme.

Nothing to Disclose: LJM, PLC, PSK

Pub # P2-768

Session Information POSTER SESSION: CLINICAL - Worldwide Efforts To Improve Endocrine Care (1:30 PM-3:30 PM)

Title A Randomized Controlled Trial Investigating Self-Weighing and the Prevention of Excess Weight Gain in Early Pregnancy

Author String C Lombard, C Harrison, H Teede
School of Public Health and Preventive Medicine, Monash University, Melbourne, Australia

Body **BACKGROUND:** Self-weighing is emerging as a potentially important strategy in weight management yet few trials have studied self-weighing in randomized controlled trials. During pregnancy excessive gestational weight gain, especially with pre-existing excess weight, increases complications including caesarean delivery, hypertension and gestational diabetes mellitus (GDM).
OBJECTIVE: To evaluate the influence of self-weighing as a key component of a behavioural intervention on weight change in early pregnancy, targeting overweight women at risk of developing GDM.
METHOD: In a randomized controlled clinical trial, women who were overweight (body mass index $\geq 25.0\text{kg/m}^2$ or $\geq 23\text{kg/m}^2$ if high risk ethnicity), <15 weeks gestation and at high risk for developing GDM based on known risk factors (age, weight, ethnicity, family history of diabetes, previous GDM) were recruited from a hospital-based clinic and randomized to the intervention ($n=106$) or control ($n=99$) group. An adapted successful self-management lifestyle intervention (HeLP-her) was delivered to the intervention group from 14 weeks gestation and regular self-weighing was emphasized as a key behavioral strategy. The control group received generic diet and physical activity guidelines for pregnancy with no instruction to self-weigh. Both groups received standard antenatal care. Measures included weight, frequency of self-weighing, physical activity and food intake at baseline and 28 weeks.
RESULTS: The mean (\pm SEM) age and BMI of women recruited was $32.0\pm 0.3\text{yrs}$ and $30.5\pm 0.42\text{kg/m}^2$ respectively, with no difference between groups. At 28 weeks regular self-weighing (daily, weekly or monthly) was associated with significantly less weight gain in intervention participants (5.69kg , 95%CI, 5.09 to 6.30) compared to the control participants (7.06kg , 95%CI, 6.02 to 8.11) a difference between groups of -1.37kg (95%CI, -2.48 to -0.25 , $p=0.02$). Women in both intervention and control groups who did not self-weigh gained a mean of 6.92kg (95% CI, 4.89 to 8.93) and 6.9kg (95% CI, 5.10 to 6.38) respectively.
Conclusion: Self-weighing when paired with a simple self-management intervention to prevent excess weight gain has a significant impact on weight change in high risk pregnancies. This work has significant public health implications.

Lombard CB et al., BMJ 2010, http://www.bmj.com/cgi/content/abstract/341/jul13_1/c3215

Sources of Research Support: BRIDGES Grant from the Global Diabetes Foundation. BRIDGES, an International Diabetes Foundation project is supported by an educational grant from Eli Lilly and Company.

Nothing to Disclose: CL, CH, HT

Pub #	CDW4-1
Session Information	CAREER DEVELOPMENT WORKSHOP: Career Development Workshop: Opportunities for International Trainees (2:00 PM - 2:45 PM)
Title	TBD
Author String	To Be Determined TBD, TBD
Body	

Pub #	CDW4-2
Session Information	CAREER DEVELOPMENT WORKSHOP: Career Development Workshop: Opportunities for International Trainees (2:00 PM - 2:45 PM)
Title	TBD
Author String	To Be Determined TBD, TBD
Body	

Pub #	NS5-1
Session Information	ENDOCRINE NURSES SYMPOSIUM: CLINICAL - Breakout Sessions (2:00 PM - 3:00 PM)
Title	Endocrine Stim Testing 101
Author String	S Carpenter Mayo Clinic, Rochester, MN
Body	Disclosure Incomplete: SC

Pub # NS5-2

Session Information ENDOCRINE NURSES SYMPOSIUM: CLINICAL - Breakout Sessions (2:00 PM - 3:00 PM)

Title Endocrine Stim Testing 101

Author String RM Hower
Lehigh Valley Hospital, Allentown, PA

Body Endocrine Tests are dynamic tests used to detect hormone imbalances in children and adults. Tests are designed to check the pituitary gland and the hormones it makes. Endocrine tests may also check growth hormone, adrenal gland function, kidney stones, bone health, blood sugar and reproductive issues. Endocrine testing nurses schedule, coordinate and conduct tests to determine excess or deficiencies in any one or more of these hormones. Endocrine tests can be divided into categories examining the function of certain organs such as the adrenal glands and thyroid, or certain diseases such as primary hyperaldosteronism, pheochromocytomas, diabetes insipidus, growth hormone deficiency or excess, hypogonadism, premature puberty, hypo- and hyperglycemia, intestinal malabsorption, kidney stone production and calcium metabolism. Tests are backed by protocols and are subject to review. Patients are scheduled for tests and receive instructions regarding necessary preparations needed for the test. Collaboration in research studies is also an important part of the role as well as mentoring medical students.

Nothing to Disclose: RMH

Pub # NS5-3

Session Information ENDOCRINE NURSES SYMPOSIUM: CLINICAL - Breakout Sessions (2:00 PM - 3:00 PM)

Title Practical Application of Yoga in Endocrine Nursing Practice

Author String B Lucasey
University of Kansas Medical Center, Kansas City, KS

Body Medicine is now becoming more and more aware of the importance of the balance between mind and body, realizing that many physical ailments have their origins in poor mental functioning as well as poor physical conditioning. Alternative therapies are fast finding a niche in the overall health management field. The reason for this are simplicity and lack of side effects.
But how do you safely advise a specific type of exercise program? Take that first step and becoming familiar with what yoga can do for you and the people that look to you for advice. This session will provide an overview of The Hatha branch of yoga with examples of balance, strength and flexibility poses.
Yoga is simple (no equipment), efficient (10 - 60 minutes sessions) and affordable (no gym membership required). Yoga can be practiced within a group setting or simply alone in the comfort and privacy of your home. Yoga is a method to come back to yourself - expand your boundaries and relax. How simple is that?

Nothing to Disclose: BL

Pub #	NS5-4
Session Information	ENDOCRINE NURSES SYMPOSIUM: CLINICAL - Breakout Sessions (2:00 PM - 3:00 PM)
Title	IV Bisphosphonates: The Role of the Endocrine Nurse
Author String	L Coppolo Basset Medical Center, Cooperstown, NY
Body	Disclosure Incomplete: LC

Pub #	NS5-5
Session Information	ENDOCRINE NURSES SYMPOSIUM: CLINICAL - Breakout Sessions (2:00 PM - 3:00 PM)
Title	The Endocrine Nurse's Role in Post-Operative Pituitary Surgery Care
Author String	MH Gurel Massachusetts General Hospital, Boston, MA
Body	Disclosure Incomplete: MHG

Pub # NS5-6

Session Information ENDOCRINE NURSES SYMPOSIUM: CLINICAL - Breakout Sessions (2:00 PM - 3:00 PM)

Title A Review of Emerging Diabetes Therapies or It's Time To Stop Flogging the Beta Cells

Author String S Hendricks
The Methodist Hospital, Houston, TX

Body

Recent government reports predict that the number of adults with diabetes could double or triple by the year 2050. The vast majority of this increase is expected to be in type 2 diabetes. In an effort to meet this challenge scientists are exploring new targets for diabetes therapeutics.

Older therapies that primarily target the failing beta cells have been limited by ineffectiveness, weight gain, poor durability and hypoglycemia. New therapies have emerged that leverage gut hormones, or incretins, for control of post-prandial glucoses without the burden of weight gain and hypoglycemia. In fact some are associated with weight loss. These agents are available in oral and injectable options. Another hormone, amylin, normally co-secreted with insulin in healthy beta cells is deficient in diabetes. When administered before meals in insulin-deficient individuals amylin has been useful in post-prandial glucose control through delaying of gastric emptying with early satiety and inhibition of glucagon secretion. An old therapy, bromocriptine, long used for treatment of hyperprolactinemia has recently been approved for treatment of type 2 diabetes. This represents a new class of antidiabetic therapy that may lower blood glucose without increasing insulin or cause significant weight gain. The exact mechanism of action is not fully understood, it is known to mimic the action of dopamine, a neurochemical messenger, at specific dopamine receptors in the brain.

We have at least three new classes of agents on the horizon. The first and closest to market is the SGLT-2 inhibitors which work in the kidney to lower the glucose threshold causing glycosuria. This loss of glucose in the urine lowers serum glucose and causes weight reduction as a result of loss of calories in the urine. Another exciting therapy in the pipeline is the glucagon receptor antagonists. This class of therapies offer the potential to decrease hepatic glucose production and lower blood glucose in patients with type 2 diabetes. A third class of therapy in the offing is the glucokinase activators (GKAs). In the liver, glucokinase mediates glucose utilization and glycogen synthesis. In the pancreas glucokinase is involved in glucose-stimulated insulin release and may have an effect in antagonizing apoptosis in B cells. As with some of our other new weapons in our glucose fighting armamentarium GKAs may have dual mechanisms of action in the pancreas and liver.

Nothing to Disclose: SH

Pub # NS5-7

Session Information ENDOCRINE NURSES SYMPOSIUM: CLINICAL - Breakout Sessions (2:00 PM - 3:00 PM)

Title Developing a Clinical Research Project in Endocrine Nursing Practice

Author String ME Eckert-Norton
State University of New York Downstate, Brooklyn, NY

Body Endocrine nursing practice presents many opportunities for clinical research. However, it is often the case that nurses working in endocrinology may need support in developing research questions and identifying appropriate methods to address these questions. In this workshop, participants will initiate a reflective process to identify potential research topics in their practice, get tips for searching for relevant literature and suggestions for methods that could be applied to the topics identified. A bibliography of tools to support the development of a research proposal will be included.

Coauthor: Patricia S. Via, NP, MS; Division of Endocrinology, McGuire Veterans Affairs Medical Center, Richmond, VA.

Disclosure Incomplete: MEE-N

Pub #	NS5-8
Session Information	ENDOCRINE NURSES SYMPOSIUM: CLINICAL - Breakout Sessions (2:00 PM - 3:00 PM)
Title	Developing a Clinical Research Project in Endocrine Nursing Practice
Author String	PS Via McGuire Veterans Affairs Medical Center, Richmond, VA
Body	Nothing to Disclose: PSV

Pub # M27

Session Information MEET-THE-PROFESSOR: CLINICAL - Management Issues in Adult Growth Hormone Replacement (2:45 PM - 3:30 PM)

Title Management Issues in Adult Growth Hormone Replacement

Author String P Chanson
Hospital Bicetre, Le Kremlin-Bicetre, France

Body Session supported by: Novo Nordisk Inc. & Pfizer, Inc.

Disclosures: PC: Advisory Group Member, Eli Lilly & Company; Clinical Researcher, Ipsen, Merck & Co., Novartis Pharmaceuticals, Novo Nordisk, Pfizer, Inc.

Pub # CMF5-1
Session Information CASE MANAGEMENT FORUM: CLINICAL - Gauging Response to Osteoporosis Therapy (2:45 PM - 3:30 PM)
Title Gauging Response to Osteoporosis Therapy
Author String CB Niewoehner
Minneapolis Veterans Affairs Medical Center, Minneapolis, MN
Body Session supported by: Amgen

Nothing to Disclose: CBN

Pub # CMF5-2
Session Information CASE MANAGEMENT FORUM: CLINICAL - Gauging Response to Osteoporosis Therapy (2:45 PM - 3:30 PM)
Title Gauging Response to Osteoporosis Therapy
Author String CJ Rosen
Maine Medical Center Research Institute, Scarborough, ME
Body Session supported by: Amgen

Nothing to Disclose: CJR

Pub #	M22
Session Information	MEET-THE-PROFESSOR: CLINICAL - Contraception across the Span of Child-Bearing Years (2:45 PM - 3:30 PM)
Title	Contraception across the Span of Child-Bearing Years
Author String	GM Christman University of Michigan, Ann Arbor, MI
Body	Nothing to Disclose: GMC

Pub #	M23
Session Information	MEET-THE-PROFESSOR: CLINICAL - Controversies in Cushing Syndrome (2:45 PM - 3:30 PM)
Title	Controversies in Cushing Syndrome
Author String	JDC Newell-Price University of Sheffield, Sheffield, UK
Body	Session supported by: Corcept Therapeutics Disclosure Incomplete: JDCN-P

Pub #	M24
Session Information	MEET-THE-PROFESSOR: CLINICAL - Disorders of Sex Development in Children (2:45 PM - 3:30 PM)
Title	Disorders of Sex Development in Children
Author String	JC Achermann University College of London Institute of Child Health, London, UK
Body	Nothing to Disclose: JCA

Pub #	M25
Session Information	MEET-THE-PROFESSOR: CLINICAL - Insulin Pumps (2:45 PM - 3:30 PM)
Title	Insulin Pumps
Author String	AL McCall University of Virginia Health System, Charlottesville, VA
Body	Session supported by: Lilly USA, LLC Nothing to Disclose: ALM

Pub #	M26
Session Information	MEET-THE-PROFESSOR: CLINICAL - Issues in the Diagnosis & Treatment of Klinefelter Syndrome (2:45 PM - 3:30 PM)
Title	Issues in the Diagnosis & Treatment of Klinefelter Syndrome
Author String	AS Dobs Johns Hopkins University School of Medicine, Baltimore, MD
Body	Nothing to Disclose: ASD

Pub #	M28
Session Information	MEET-THE-PROFESSOR: CLINICAL - Treatment of Severe Thyrotoxicosis (2:45 PM - 3:30 PM)
Title	Treatment of Severe Thyrotoxicosis
Author String	MT McDermott University of Colorado Hospital, Denver, CO
Body	Nothing to Disclose: MTM

Pub #	NS6-1
Session Information	ENDOCRINE NURSES SYMPOSIUM: CLINICAL - Adult Growth Hormone Deficiency (3:15 PM - 4:00 PM)
Title	Adult Growth Hormone Deficiency
Author String	L Katznelson Stanford University, Stanford, CA
Body	Disclosure Incomplete: LK

Pub # S32-1

Session Information SYMPOSIUM SESSION: BASIC - Actions of Growth Hormone in Neurogenesis (3:45 PM - 5:15 PM)

Title Growth Hormone & Ghrelin Induce Adult Neurogenesis

Author String J Isgaard
Göteborg University, Göteborg, Sweden

Body Accumulating evidence suggest that the GH/IGF-I axis is involved in the regulation of brain growth, development and myelination. In addition, both GH and IGF-I affect cognition and biochemistry in the adult brain. Some of the effects of GH are probably mediated by circulating IGF-I, while other effects may be due to locally produced IGF-I within the brain. However, in other aspects, it seems that GH acts directly without involving IGF-I (either circulating or locally).
Plasticity in the central nervous system (CNS) may be viewed as changes in the functional interplay between the major cell types neurons, astrocytes and oligodendrocytes. GH and IGF-I affect all three of these cell type in several aspects. Apart from neuroprotective effects of GH and IGF-I in different experimental models of CNS injury, IGF-I has been found to increase progenitor cell proliferation and new neurons, oligodendrocytes and blood vessels in the dentate gyrus of the hippocampus. It appears that the MAPK signaling pathway is required for IGF-I-stimulated proliferation *in vitro*, whereas the PI3K/Akt or MAPK/Erk signaling pathway appears to mediate antiapoptotic effects.
Ghrelin has also been found to be a neuroactive peptide. The hippocampus expresses the GHS-R1a and experimental data suggest that ghrelin influences several biochemical processes in the hippocampus including increased memory retention in rats and enhanced spatial learning and memory. Ghrelin has also been shown to promote neurogenesis in the dorsal motor nucleus of the vagus in adult rat, both *in vitro* and *in vivo*. Recent studies suggest that both ghrelin and synthetic analogues have neuroprotective effects. We have shown in a neonatal rat model with experimental unilateral hypoxic-ischemic (HI) injury, that intracerebroventricular (icv) injections of hexarelin significantly reduced the area of injury in various parts of the brain and the most pronounced effect was found in the hippocampus. Moreover, the protective effects of hexarelin were associated with signaling through the PI3K/Akt pathway and reduced caspase 3 activity. We have also reported proliferative and protective effects of ghrelin and hexarelin on adult hippocampal progenitor (AHP) cells from rat.
In summary, these findings suggest a number of interactions between components of the GH/IGF-I system and the brain and offer interesting perspectives on their potential therapeutic role after brain injury and degenerative disease.

Session supported by: Novo Nordisk Inc. & Pfizer, Inc.

Nothing to Disclose: JI

Pub # S32-2

Session Information SYMPOSIUM SESSION: BASIC - Actions of Growth Hormone in Neurogenesis (3:45 PM - 5:15 PM)

Title Growth Hormone Signaling & Neurogenesis

Author String AM Turnley
University of Melbourne, Melbourne, Australia

Body Growth hormone (GH) has numerous effects in the body and is most commonly known for its role in regulating metabolism and body growth. However, GH, the GH receptor and a major regulator of the GH signalling pathway, suppressor of cytokine signalling 2 (SOCS2), are also expressed in the developing and adult nervous system.

During development, decreasing or increasing GH signalling by use of GH receptor null, SOCS2 overexpressing transgenic and SOCS2 null mice, has reciprocal effects on cortical neurogenesis and neuronal specification. SOCS2 null mice have increased GH signalling and fewer neurons in their cortex, while GH receptor null mice and SOCS2 transgenic mice have decreased GH signalling and more neurons (1,2). GH inhibits neurogenesis in the neural precursors by decreasing expression of the neurogenic transcription factor Neurogenin-1 (3). Overexpression of SOCS2 blocks the inhibitory effect of GH and promotes neurogenesis. Altering GH signalling in these mice also changes types of neurons made, with differences in the composition of inhibitory interneuron subpopulations in the cortex (1,2).

In the adult, there are two brain regions that continue to produce neurons, the subventricular zone (SVZ) of the lateral ventricles and the dentate gyrus of the hippocampus. Interestingly, effects of GH and SOCS2 may be at least partly disconnected in adult neural precursor cells in these two regions. In vitro, GH promotes proliferation of neural precursor cells from the SVZ and delays their neuronal differentiation (4), which is similar to effects observed during development. Conversely, in the adult hippocampus, neither GH nor SOCS2 has an effect on endogenous neural precursor proliferation or neuronal differentiation. However, SOCS2 but not GH regulates long term survival of the newborn neurons, indicating a GH independent effect of SOCS2 (5). This may be related to the ability of SOCS2 to promote neurite outgrowth of neurons (6,7), an effect which does not involve GH (8) and which may increase the chance of newborn neurons to integrate into existing circuitry in the adult brain.

Overall, GH and a major regulator of GH signalling, SOCS2, play multiple roles in regulation of neural precursor cell proliferation, neurogenesis and neuronal differentiation. Perturbations in this system correlate with effects on cognition and memory and other aspects of neurophysiology observed in people with growth phenotypes, aging and following brain injury (9,10).

Session supported by: Novo Nordisk Inc. & Pfizer, Inc.

- (1) Ransome et al., Eur. J. Neurosci 2004; 19:2069
- (2) Ransome and Turnley, Neuroscience 2005; 132:673
- (3) Turnley et al., Nature Neuroscience 2002; 5:1155
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- (5) Ransome and Turnley, Hippocampus 2008; 18:1034
- (6) Goldshmit et al., J Biol Chem 2004; 279:16349
- (7) Goldshmit et al., Eur J Neurosci 2004; 20:2260
- (8) Scott et al., Brain Res 2006; 1067:138
- (9) Turnley, Trends Endocrinol Metab 2005; 16:53
- (10) Turnley, Pediatr Endocrinol Rev 2005; 2:366

Sources of Research Support: National Health and Medical Research Council of Australia, ANZ Charitable Trusts, Clive and Vera Ramaciotti Foundation.

Nothing to Disclose: AMT

Pub # S32-3

Session Information SYMPOSIUM SESSION: BASIC - Actions of Growth Hormone in Neurogenesis (3:45 PM - 5:15 PM)

Title Growth Hormone Actions in Retinal Development

Author String S Harvey
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Body The neural retina is rarely considered as a target site of growth hormone (GH) action, but pituitary GH excess is associated with retinopathy, while pituitary GH deficiency is associated with optic nerve hypoplasia, refraction, retinitis pigmentosa, glaucoma and other ocular diseases. This suggests actions of pituitary GH in retinal function, even though the blood-ocular barriers are thought to impede the entry of systemic GH into the eye. The retina is, however, an extrapituitary site of GH production and retinal GH acts as an autocrine or paracrine neurotrophic factor during development. Retinal GH, for instance, promotes the *in vitro* and *in vivo* survival of retinal ganglion cells (RGCs) during early neurogenesis in the chick embryo, since GH immunoneutralization or GH gene knockdown stimulates retinal cell death. Retinal GH may also promote RGC survival in elderly human adults, as GH is not present in any apoptotic RGCs in post-mortem retinal tissues, but is present in most non-apoptotic RGCs. Increased RGC death in diabetic patients with retinopathy may similarly reflect the low vitreous GH levels in the vitreous of these patients. GH may also be required for retinal cell proliferation, since the neuroblastic, inner plexiform and ganglion cells layers of the retina are reduced in size in GHR receptor (GHR) deficient mice, possibly reflecting their decreased expression of BASP (brain-abundant-membrane attached signal protein)-1, which stimulates nerve growth. Retinal GH has also been shown to stimulate RGC neurite outgrowth in early chick embryos. GH is also thought to induce retinal angiogenesis, by direct actions mediated through retinal GHRs and indirectly through its induction of hyperglycemia in the periphery. Pituitary GH and/or retinal GH therefore have numerous endocrine, paracrine or autocrine actions in the neural retina during development.

Session supported by: Novo Nordisk Inc. & Pfizer, Inc.

Nothing to Disclose: SH

Pub # S33-1

Session Information SYMPOSIUM SESSION: BASIC - Extra-Nuclear Steroid Receptor Signaling: Is It Safe To Go into the Membrane? (3:45 PM - 5:15 PM)

Title From the outside Looking in: Membrane to Nuclear Signaling by Steroids

Author String SR Hammes
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Body Steroids are known to regulate signaling both inside and outside of the nucleus. The current consensus suggests that these extranuclear, or nongenomic, steroid-triggered signals in large part function to enhance intranuclear, or transcriptional, steroid-mediated signals. To understand how these two signaling processes integrate with one another, we have focused on androgen-mediated signaling in two seemingly disparate systems: *Xenopus laevis* oocytes and prostate cancer cells. We initially focused on androgen-triggered oocyte maturation (meiotic resumption) in *Xenopus* oocytes, since this is perhaps the only biologically relevant steroid-mediated process that occurs completely independent of transcription. Our goal was to use this [ldquo; simple[rldquo] model system to characterize novel nongenomic androgen-mediated signaling pathways that could then be examined in the more disease-related prostate cancer model. In fact, as we will demonstrate, we have been fortunate in that we were able to do exactly what we planned. We will focus on paxillin, a scaffolding molecule primarily thought to regulate cytoplasmic kinase signaling and cytoskeletal functions. We will provide evidence that paxillin in fact plays a critical role of a liaison between between extranuclear and nuclear androgen-mediated signaling. In fact, we will show that paxillin's importance extends beyond that of a regulator of steroid signaling, as it may be a general regulator of Erk-mediated transcription regardless how Erk signaling is activated. Finally, we will provide evidence that paxillin may be an important regulator of prostate cancer growth both in-vitro and in human disease.

Sources of Research Support: NIH Grant: DK059913.

Nothing to Disclose: SRH

Pub # S33-2

Session Information SYMPOSIUM SESSION: BASIC - Extra-Nuclear Steroid Receptor Signaling: Is It Safe To Go into the Membrane? (3:45 PM - 5:15 PM)

Title Mechanisms of Rapid Aldosterone Action

Author String BJ Harvey
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Body The steroid hormone aldosterone is a key regulator of ion transport in the kidney and contributes to homeostatic whole body electrolyte balance. Aldosterone can also produce deleterious structural changes in tissues by inducing hypertrophy and the dysregulation of proliferation and apoptosis, leading to the development of renal and cardiovascular pathologies (1). Aldosterone exerts both rapid non-genomic and latent genomic actions principally through the mineralocorticoid receptor (MR). The Aldosterone-MR complex may act as a ligand-dependent transcription factor or rapidly through activation of protein kinases and trans-activation of other receptors such as EGFR (2). Rapid cellular actions of aldosterone occur within seconds to minutes, do not involve transcription or translation and can modulate directly or indirectly the later genomic responses. This review lecture describes current knowledge regarding the mechanisms and targets of rapid aldosterone action in epithelial tissues of the kidney and colon and in non-epithelial tissues such as the brain and cardiovascular system. We also discuss the involvement of aldosterone-mediated rapid signalling cascades in the development of chronic kidney disease and heart failure, and the antagonists that can inhibit these pathophysiological responses.

(1) Dooley R, Harvey BJ, Thomas W. The regulation of cell growth and survival by aldosterone. *Front Biosci* 2011 Jan 1;16:440-57. PubMed PMID: 21196180

(2) Thomas W, Harvey BJ. Mechanisms Underlying Rapid Aldosterone Effects in the Kidney. *Annu Rev Physiol*. 2011; Feb 19; 73: [Epub ahead of print] PubMed PMID: 20809792.

Additional abstract authors: Ruth Dooley, Warren Thomas

Sources of Research Support: Science Foundation Ireland Research Frontiers Programme.

Nothing to Disclose: BJH

Pub # S33-3

Session Information SYMPOSIUM SESSION: BASIC - Extra-Nuclear Steroid Receptor Signaling: Is It Safe To Go into the Membrane? (3:45 PM - 5:15 PM)

Title Mechanisms of Rapid Progesterone Receptor Signaling in Breast Cancer

Author String CA Lange
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Body Progesterone receptors (PR) are critical mediators of mammary gland development and contribute to breast cancer progression. Newly defined cytoplasmic or [ldquo]rapid[rdquo] signaling actions of nuclear receptors, including PR, are functionally linked to their genomic actions. For example, progestin-induced rapid activation of cytoplasmic protein kinases leads to selective regulation of growth-promoting genes by phospho PR species. Herein, we define a new signaling [ldquo]common docking[rdquo] (CD) domain located within the PR-B upstream segment (BUS). The presence of CD in BUS confers PR-B isoform-specific interactions with components of cytoplasmic signaling pathways, including MAP Kinase Phosphatase-3 (MKP3) and Ck2 a protein kinase required for cell survival and commonly overexpressed in breast and other cancers. Herein, we show that the CD domain mediates Ck2-dependent PR-B Ser81 phosphorylation. Notably, endogenous phosphorylation of PR Ser81 is ck2-dependent and progestin-regulated in intact cells, but also occurs in the absence of PR ligands, when cells enter the G1/S phase of the cell cycle. T47D breast cancer cells stably expressing a PR-B mutant that cannot be phosphorylated at Ser81 (S81A) formed fewer soft agar colonies. Regulation of selected genes by PR-B, but not PR-A, also required Ser81 phosphorylation for basal and/or progestin-regulated (BIRC3, HSD11 β 2, and HbEGF) expression. Additionally, wt PR-B, but not S81A mutant PR, was robustly recruited to a PRE-containing transcriptional enhancer region of BIRC3; abundant ck2 also associated with this region in cells expressing wt but not S81A PR. We conclude that phospho-Ser81 PR provides a platform for ck2 recruitment and regulation of selected PR-B target genes; this can occur independently of ligand in dividing breast cancer cells. Understanding how PR functions in the context of high kinase activities characteristic of breast cancer is critical to understanding the basis of tumor-specific changes in gene expression and will speed the development of highly selective treatments.

Sources of Research Support: NIH/NCI R01 CA123763 (formerly R01 DK53825-10; NIH/NIDDK).

Nothing to Disclose: CAL

Pub # S34-1
Session Information SYMPOSIUM SESSION: BASIC - Molecular Mechanisms Underlying Coordinated Control of Energy Balance (3:45 PM - 5:15 PM)
Title Prolyl/Carboxypeptidase Regulation of Food Intake through Melanocortin Degradation
Author String S Diano
Yale University Medical School, New Haven, CT
Body Nothing to Disclose: SD

Pub # S34-2

Session Information SYMPOSIUM SESSION: BASIC - Molecular Mechanisms Underlying Coordinated Control of Energy Balance (3:45 PM - 5:15 PM)

Title Proopiomelanocortin Processing & Regulation of Energy Balance: Role of FOXO1 & Carboxypeptidase E

Author String SL Wardlaw
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Body Hypothalamic proopiomelanocortin (POMC) neurons play a key role in regulating energy balance and neuroendocrine function. The POMC precursor protein undergoes extensive posttranslational processing that is essential for the generation of biologically active MSH peptides as well as the endogenous opioid peptide, β -endorphin (β -EP). This involves several processing enzymes, including prohormone convertase (PC)1/3, PC2 and carboxypeptidase E (CPE). α -MSH and β -EP have opposing biological actions in several systems mediated by distinct melanocortin and opioid receptor pathways. α -MSH can attenuate the neuroendocrine effects of β -EP in rodents and primates. With respect to feeding, α -MSH is inhibitory while β -EP has stimulatory effects. Furthermore, we have recently shown that β -EP can attenuate the effects of α -MSH on energy balance. β -EP 1-31 can be cleaved by CPE to β -EP 1-27 and 1-26 which have markedly reduced opioid activity. We have recently shown that CPE may link FoxO1 (a transcription factor that mediates effects of insulin) in POMC neurons with regulation of food intake (1). Mice with FoxO1 ablation in POMC neurons have a lean phenotype with decreased food intake and increased Cpe expression in the arcuate. Analysis of POMC peptides in these mice revealed an anorexigenic profile with a selective increase of α -MSH and HPLC analysis demonstrated relatively more β -EP 1-27 and β -EP 1-26 compared to β -EP 1-31; this is opposite to the pattern seen in CPE-deficient mice. Regulated CPE-dependent cleavage of α -MSH and β -EP could thus contribute to the phenotype of Pomc-FoxO1^{-/-} mice by both increasing α -MSH levels and reducing opioid activity. However CPE-dependent sorting of POMC to the regulated secretory pathway may also play a role in this process. FoxO1 has been shown to directly interact with and regulate Cpe and there is evidence that Cpe expression in the arcuate is downregulated by chronic diet-induced obesity. Furthermore the initial adaptive response to high fat feeding in mice, that is known to involve the melanocortin system, is associated with changes in β -EP processing that would serve to limit weight gain. These studies link the insulin signaling pathway to CPE-dependent POMC processing and provide additional evidence that POMC processing is regulated with respect to energy balance.

(1) Plum L, et al., Nature Medicine 2009; 15:1195

Sources of Research Support: NIH Grant DK080003.

Nothing to Disclose: SLW

Pub # S34-3

Session Information SYMPOSIUM SESSION: BASIC - Molecular Mechanisms Underlying Coordinated Control of Energy Balance (3:45 PM - 5:15 PM)

Title Role of GABAergic & Glutamatergic Neurotransmission in Regulating Feeding Behavior

Author String BB Lowell
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Body Leptin Action and the Role of GABAergic Neurons:
Uncertainty exists regarding the leptin-responsive, first-order neurons which mediate leptin's anti-obesity effects, and this has made it difficult to determine the neurobiological basis of leptin action. A role for fast-acting neurotransmitters has been underappreciated; focus has been primarily on neuropeptide-expressing neurons. Using Cre/Lox technology, we have investigated whether first-order neurons are inhibitory (GABAergic, using VGAT-ires-Cre knockin mice) or excitatory (glutamatergic, using VGLUT2-ires-Cre knockin mice). Remarkably, leptin's anti-obesity effects are mediated predominantly by GABAergic neurons; glutamatergic neurons play only a minor role. Leptin, working directly on presynaptic GABAergic neurons, many of which appear not to express AgRP, reduces inhibitory tone to postsynaptic POMC neurons. As POMC neurons prevent obesity, their disinhibition by leptin action on presynaptic GABAergic neurons likely mediates, at least in part, leptin's anti-obesity effects.

AgRP Neurons and the Role of Glutamatergic Input:
Importance of AgRP Neurons: Using DREADD technology (Designer Receptors Exclusively Activated by Designer Drugs), we have created mice in which the activity of AgRP neurons can be specifically, rapidly and reversibly controlled. Acute activation of AgRP neurons, even in mice that are fully sated, rapidly and dramatically induces feeding. In addition, this greatly increases motivation to work for food and also causes intense [ldquo]food-seeking[rdquo] activity. On the other hand, acutely inhibiting AgRP neurons in hungry mice markedly reduces feeding. Thus, AgRP neuron activity is both necessary and sufficient for feeding. It will be important to understand the neuronal, subcellular and molecular mechanisms controlling AgRP neuron activity.

Role of Glutamatergic Input: NMDA-receptors bind glutamate, are ionotropic (allow influx of Na⁺ and Ca²⁺), and, importantly, are key mediators of activity-dependent plasticity of glutamatergic synapses. By deleting NR1 (subunit required for NMDA-R function), we have found that NMDA-Rs on AgRP neurons, but not NMDA-Rs on POMC neurons, play a key role in regulating feeding. Consistent with this, we have observed that AgRP neurons, but not POMC neurons, have numerous dendritic spines - the subcellular [ldquo]communication hubs[rdquo] which receive excitatory synapses. This likely has important implications for the regulation and plasticity of feeding behavior.

Additional Authors:
Lowell Lab (in alphabetical order): Shuichi Koda, Dong Kong, Michael Krashes, Tiemin Liu, Linh Vong, Chianping Ye.
Also from BIDMC: Eleftheria Maratos-Flier, Andrew Adams.
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From Univ. of North Carolina: Bryan Roth, Sarah Rogan.

Nothing to Disclose: BBL

Pub # S35-1

Session Information SYMPOSIUM SESSION: TRANSLATIONAL - Advances in Adrenal Neoplasia (3:45 PM - 5:15 PM)

Title Aberrant Receptors in Primary Aldosteronism

Author String A Lacroix
Centre de Recherche du Centre Hospitalier de l'Université de Montréal, Montreal, Canada

Body Primary aldosteronism (PA) is the most common secondary cause of hypertension with recent evidence that it affects approximately 10% of all hypertensive patients; it is also associated with higher prevalence of cardiovascular morbidities compared to essential hypertension. The mechanisms responsible for the renin-independent regulation of aldosterone secretion in PA are poorly understood. In ACTH-independent Cushing's syndrome, cortisol secretion is frequently regulated by the aberrant expression of G-protein coupled receptors (GPCRs) in unilateral adenomas and in bilateral macronodular adrenal hyperplasia. By analogy, some recent studies also indicate aberrant expression or increased function of several GPCR as a potential cause for excess aldosterone production in some aldosteronomas (APA) and in bilateral idiopathic hyperaldosteronism (IHA). Initial studies in resected APA have used *in vitro* assays of receptor expression levels, while the aberrant regulation of aldosterone secretion *in vivo* was not systematically assessed. This included demonstration of increased expression of receptors in some cases for ACTH (MC2R), serotonin (HTR4), angiotensin-II (AGTR1), LH (LHCGR), GnRH (GNRHR), and TSH (TSHR). In recent cases or small series, systematic evaluation of aberrant regulation of aldosterone secretion by various tests revealed a high percentage of renin and ACTH-independent increased stimulation by upright posture, 5-HT4 agonists, vasopressin, ACTH 1-24, and in some cases by LHRH, LH, GIP, glucagon, isoproterenol or TRH both in APA and IHA. More than one aberrant response can be found in the same patient and in some cases aberrant co-secretion of small amounts of cortisol can be found simultaneously. The prevalence of aberrant hormone receptors in PA appears to be elevated in initial small series, but larger systematic studies are required to establish its true frequency. The variable levels of expression and function of aberrant receptors may underlie fluctuations of aldosterone and aldosterone/renin ratios depending on posture, endogenous ACTH levels, saline infusion, prandial state and in adrenal vein sampling before or after ACTH 1-24 administration. The functional role of aberrant GPCR offers the potential of novel pharmacological therapies to block the aberrant receptors with specific antagonists particularly in patients requiring medical therapy for bilateral IHA.

Co-authors: Livia M. Mermejo, Solange Grunenwald, T[acirc]nia L. Mazzuco, Sylvie Oble and Isabelle Bourdeau

Nothing to Disclose: AL

Pub # S35-2

Session Information SYMPOSIUM SESSION: TRANSLATIONAL - Advances in Adrenal Neoplasia (3:45 PM - 5:15 PM)

Title Therapeutic Advances in Adrenocortical Cancer

Author String M Fassnacht
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Body Adrenocortical carcinoma (ACC) is a rare malignancy and most of the therapeutic strategies are not validated prospectively yet. However, significant improvement has been made in the last few years. Open adrenalectomy is treatment of choice for respectable tumors. However, two recent retrospective studies provided evidence that a laparoscopic approach might be also a safe alternative in selected cases (especially in tumors preoperatively of uncertain malignancy). Based on a study by Terzolo et al. (NEJM 2007), adjuvant mitotane treatment is recommended for the majority of patients. Patients on high risk for recurrence (e.g. after R1 resection) might benefit from additional radiotherapy of the tumor bed. However, prospective data suggest that the outcome in patients with stage II ACC is better as assumed in the past. Therefore, prospective trials like the currently running randomized ADIUVO trial on adjuvant mitotane are urgently needed. In advanced ACC, the first ever randomised trial (FIRM-ACT study) will set the new standard in first-line cytotoxic therapy. Although preliminary results showed only a trend for overall survival favoring the combination of etoposide, doxorubicin, cisplatin, and mitotane (EDP-M) in comparison to streptocotozin and mitotane (14.8 vs. 12.0 months; p=0.069), the progression-free survival was clearly improved by EDP-M (5.7 vs. 2.1 months; p<0.0001). In 2010, a new protocol for second and third line therapy was suggested. The combination of metronomic capecitabine and gemcitabine led to disease control for > 4 months in 13 of 28 patients (47%). In contrast to earlier reports, ACC is not radio-resistant. Therefore, palliative radiotherapy may be used in symptomatic metastatic lesions. The first experience using targeted therapies is disappointing. gefitinib, sorafenib, erlotinib+gemcitabine, or bevacizumab+capecitabine exhibited only limited efficacy in pretreated patients. However, trials testing IGF-receptor inhibitors or sunitinib are ongoing and will hopefully hold more promise. Of note, there are hints that mitotane strongly induces CYP3A4 leading to an increased metabolism of other drugs (e.g. sunitinib) diminishing possibly the effects of these compounds. Overall the prognosis of ACC is still poor and 5-year survival rate is about 45%. However, recent collaborative efforts (e.g. by the European adrenal network ENSAT) and ongoing international trials will further advance the field in the near future.

Sources of Research Support: German Ministry of Research and German Cancer Aid.

Nothing to Disclose: MF

Pub #	S35-3
Session Information	SYMPOSIUM SESSION: TRANSLATIONAL - Advances in Adrenal Neoplasia (3:45 PM - 5:15 PM)
Title	Pheochromocytoma Susceptibility Genes
Author String	PL Dahia University of Texas Health Science Center, San Antonio, TX
Body	Nothing to Disclose: PLD

Pub # S36-1

Session Information SYMPOSIUM SESSION: TRANSLATIONAL - Humanin & the Emerging Field of Mitochondrial Peptides (3:45 PM - 5:15 PM)

Title Humanin, Related Peptides & the Expanding Role of the Mitochondria

Author String P Cohen
Mattel Children's Hospital/University of California, Los Angeles, CA

Body Mitochondria are involved in energy metabolism and apoptosis, and are central to the pathogenesis of multiple diseases, including diabetes, cancer, neurodegeneration and aging. Mitochondria contain nearly a thousand proteins of nuclear origin, but the mitochondrial-chromosome only encodes 13 proteins. In 2001, humanin; a novel 24-amino-acid peptide proposed to be encoded from the 16S ribosomal RNA region of the mtDNA, was described to be a potent neurosurvival factor. Soon afterwards humanin was shown to bind and antagonize Bax and IGF1R (1). Humanin has been shown to be cytoprotective in vitro and in vivo in models of stroke, ALS, and Alzheimer's and has metaboloprotective activities against both type-1 & type-2 diabetes (2, 3) and atherosclerosis (4). We recently developed a humanin ELISA assay and demonstrated that humanin declines with aging and its levels are altered in states of insulin resistance, diabetes, endothelial dysfunction and neurodegeneration. We recently identified an additional six peptides encoded from ORFs within the 16S rRNA, which we named SHLPs (small humanin-like peptides). Analysis of their expression reveals that they are transcribed in the mitochondria from mtDNA, are detectable in plasma, and exhibit a tissue-specific distribution. SHLPs 1-5 act as potent bioactive molecules acting to induce cell survival and ROS inhibition (like humanin, via activation of Erk and Stat3 phosphorylation) but with different temporal profiles, suggesting that these peptides may act in concert. SHLP6 has opposing actions, potentially inducing apoptosis. These observations reveal that the mitochondria possess previously unappreciated roles in the regulation of metabolism and apoptosis that occur via the synthesis of mitochondrial-derived peptides (MDPs). We propose that the mitochondrial peptidome could explain important new aspects of mitochondrial biology and dysfunction with relevance to human biology and disease and that the novel MDPs we describe here may represent retrograde communication signals from the mitochondria.

Pinchas Cohen, MD and Laura Cobb, PhD; UCLA

1. Ikonen et al. PNAS. 2003; 100:13042-13047.
2. Hoang et al. Metabolism. 2010; 59:343-349.
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Nothing to Disclose: PC

Pub # S36-2

Session Information SYMPOSIUM SESSION: TRANSLATIONAL - Humanin & the Emerging Field of Mitochondrial Peptides (3:45 PM - 5:15 PM)

Title Humanin & Testes Biology

Author String CCL Wang
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Body Humanin (HN) is a 24 amino-acid pro-survival peptide that is expressed in immature and mature rodent testes as well as in human spermatocyte and spermatids. We have shown that intratesticular HN administration reduces germ cell apoptosis induced by gonadotropin releasing hormone antagonist (GnRH-A) in rats, suggesting the possibility that HN restrains GnRH-A's pro-apoptotic action (1). Furthermore, HN reduces spontaneous germ cell apoptosis in serum-free *ex-vivo* cultures of rat seminiferous tubules. IGFBP-3 is a binding partner of HN and induces apoptosis of germ cells in the testis. HN mitigates pro-apoptotic actions of IGFBP-3 on male germ cells in the *ex-vivo* seminiferous tubules. Another intracellular binding partner of HN is BAX. *In vitro*, HN binds to BAX and prevents its translocation into the mitochondria to induce the caspase cascade and apoptosis. We recently found that HN also binds to the pro-apoptotic molecule BAX in the cytoplasm of testicular cell homogenates, suggesting HN may not only negatively regulate IGFBP-3, but also BAX (2). As precedent for this, we previously demonstrated that the balance between BAX and BCL-2, an anti-apoptotic protein, is critical in dictating the frequency of germ cell apoptosis. We have also shown that BAX partners with IGFBP-3 in testis mitochondria *in vivo* and *in vitro*, suggesting these two pro-apoptotic molecules may function together in germ cells. Together, our published and preliminary findings suggest that HN prevents cell death in the testis by harnessing the actions of IGFBP-3 and BAX. We are studying whether HN functions by activating both membrane receptor-dependent and -independent pro-survival mechanisms. We propose the HN is a key regulator of testicular germ cell apoptosis. We speculate that perturbation of HN action has conserved effects on germ cell apoptosis from rodents to humans and that future studies may show that HN is a potential therapeutic target for development for male infertility, male contraception and germ cell cancer.

1. Lue Y, Swerdloff R, Liu Q, Mehta H, Hikim AS, Lee KW, et al. Opposing roles of insulin-like growth factor binding protein 3 and humanin in the regulation of testicular germ cell apoptosis. *Endocrinology*. 2010; 151:350-7
2. Jia Y, Lee KW, Swerdloff R, Hwang D, Cobb LJ, Sinha Hikim A, et al. Interaction of insulin-like growth factor-binding protein-3 and BAX in mitochondria promotes male germ cell apoptosis. *J Biol Chem*. 2010; 285:1726-32

Nothing to Disclose: CCLW

Pub # S36-3

Session Information SYMPOSIUM SESSION: TRANSLATIONAL - Humanin & the Emerging Field of Mitochondrial Peptides (3:45 PM - 5:15 PM)

Title Metabolic Effects of Humanin

Author String R Muzumdar
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Body Humanin (HN), a novel mitochondria-associated peptide was originally identified in surviving neurons of a patient with Alzheimer's disease. Since its identification, HN has been shown to have a broad cyto-protective profile and protect against AD related neurotoxicity in neuronal and smooth muscle vascular cells, stroke, and to attenuate memory loss in AD and scopolamine-induced models. Consistent with its cyto-protective effects, we showed that HNG is cardio-protective in the setting of myocardial ischemia reperfusion. Protection against neuronal cell death by HN results from its interaction with cytokine receptor complex, CNTFR- α /WSX-1/gp130. HN analogs created by molecular manipulations of HN such as HNG (S14G-HN) are more potent than native HN in its biological actions. We demonstrated recently that Humanin, when infused intra-cerebroventricularly, significantly improves hepatic and peripheral insulin action. The central effects of HN on insulin action are associated with activation of hypothalamic STAT-3 signaling; effects that are negated by co-inhibition of hypothalamic STAT-3. Furthermore, endogenous IGFBP-3 in the hypothalamus tempers the effects of HN on glucose metabolism. This is demonstrated by the significantly higher insulin-sensitizing effects of HN analogues that do not bind IGFBP-3. Peripheral infusions of novel HN derivatives reproduce the insulin-sensitizing effects of central HN during insulin clamp. A single injection of HNGF6A significantly decreased blood glucose levels in Zucker diabetic fatty rats. Humanin analogs also increase insulin secretion both in vivo and in vitro. We conclude that humanin has potent effects on glucose homeostasis and may represent a novel link between diabetes and neuron-degeneration.

Nothing to Disclose: RM

Pub # S37-1

Session Information SYMPOSIUM SESSION: TRANSLATIONAL - Novel Influences on Hypothalamic Control of Reproductive Function in Females (3:45 PM - 5:15 PM)

Title The Metabolic Control of Reproductive Function: Leptin, Kisspeptins & beyond

Author String M Tena-Sempere
University of Cordoba, Cordoba, Spain

Body Reproduction, especially in the female, is a metabolic-challenging function that requires threshold fuel stores to proceed. Accordingly, puberty onset and fertility are influenced by the amount of body energy reserves and metabolic cues. The neuroendocrine mechanisms and signals responsible for such a tight coupling between energy homeostasis and reproduction have been partially exposed in recent years. As major milestone in this area, in mid-90's, the adipose signal of energy sufficiency, leptin, was demonstrated as essential player for the metabolic gating of reproduction acting mainly at the hypothalamus. More recently, the reproductive roles of other metabolic hormones, such as insulin and ghrelin, have been documented, and much knowledge has been gained on the ultimate pathways whereby the actions of these peripheral regulators are transmitted onto GnRH neurons. In this context, a major breakthrough has been the recognition of the putative roles of Kiss1/kisspeptin neurons, indispensable elements of the circuits governing reproductive function, as conduits for the metabolic modulation of puberty onset and fertility. In this presentation, we will provide a synoptic account of the experimental evidence suggesting the function of Kiss1 neurons as neuroendocrine integrators bridging energy balance and reproduction. Yet, supporting data from expression and pharmacological studies will be confronted with recent evidence from mouse models of genetic inactivation of leptin signaling in Kiss1 neurons. In addition, mention will be made of recent developments concerning the identification of novel molecular mechanisms for the central control of reproductive function by metabolic signals, such as mTOR, and the putative roles of various hormones, co-transmitters and neuropeptides, such as neurokinin B, in the metabolic control of hypothalamic Kiss1 signaling. Finally, the roles of additional pathways, such as those involving nesfatin-1 or the ventral premammillary nucleus, in the metabolic gating of puberty will be discussed. All in all, the data reviewed here will help to provide a mechanistic insight on how various central and peripheral signals converge and interplay to finely regulate reproductive maturation and function; mechanisms that may explain, at least in part, the basis for the well-known alterations of puberty and fertility linked to conditions of disturbed energy balance, from anorexia nervosa to morbid obesity.

Sources of Research Support: Grant BFU-2008-00984 (Ministerio de Ciencia e Innovacion, Spain), Project P08-CVI-03788 (Junta de Andalucia, Spain), and EU research contract DEER FP7-ENV-2007-1.

Nothing to Disclose: MT-S

Pub # S37-2

Session Information SYMPOSIUM SESSION: TRANSLATIONAL - Novel Influences on Hypothalamic Control of Reproductive Function in Females (3:45 PM - 5:15 PM)

Title Role of Insulin in Female Reproductive Function

Author String FP Pralong
University Hospital, Lausanne, Switzerland

Body Insulin has been shown to participate in the long-term regulation of satiety, and it is emerging as a metabolic modulator of the activity of the neuroendocrine reproductive axis as well. Indeed, mice lacking the expression of brain insulin receptors exhibit central hypogonadism and infertility. We have also shown that in normal adult mice, the administration of insulin in the periphery can stimulate LH secretion. In vitro data suggest that this effect of insulin may be mediated at the level of the hypothalamus, since insulin can also directly stimulate GnRH secretion from hypothalamic neurons in culture. In further studies performed in lean PCOS patients and normal female controls, we could demonstrate that insulin modulates LH pulsatility in the normal controls. In contrast, it did not affect the pattern of LH secretion in lean PCOS patients, who exhibited increased LH pulsatility at baseline compared to the normal control volunteers. Taken together, these data establish insulin as a peripheral metabolic signal to the neuroendocrine reproductive axis. It thus participates in a network of metabolic factors that also comprise leptin and ghrelin, which are involved in the fine tuning of the reproductive system by metabolic or nutritional changes.

Nothing to Disclose: FPP

Pub # S37-3
Session Information SYMPOSIUM SESSION: TRANSLATIONAL - Novel Influences on Hypothalamic Control of Reproductive Function in Females (3:45 PM - 5:15 PM)
Title Genetic Susceptibility to Hypothalamic Amenorrhea
Author String N Pitteloud
University of Lausanne, Lausanne, Switzerland
Body Nothing to Disclose: NP

Pub # S38-1
Session Information SYMPOSIUM SESSION: TRANSLATIONAL - Putting on the Brakes/Breaks in Prostate Cancer (3:45 PM - 5:15 PM)

Title Double-Edged Sword of Androgen-Induced DNA Double Strand Breaks in Prostate Cancer

Author String S Yegnasubramanian
Johns Hopkins University, Baltimore, MD

Body An emerging model of transcriptional activation suggests that induction of transcriptional programs, for instance by stimulating prostate cells with androgens, involves formation of DNA damage, including double strand breaks (DSB), recruitment of DSB repair proteins, and movement of newly activated genes to transcriptional hubs (1-6). The DSB can be mediated by the class II topoisomerase TOP2B, which is recruited with the androgen receptor (AR) and estrogen receptor (ER) to regulatory sites on target genes and is apparently required for their efficient transcriptional activation(2, 3). These DSB are recognized by the DNA repair machinery, triggering the recruitment of repair proteins such as PARP1, ATM and DNA-PK (2, 3). If illegitimately repaired, such DSB can seed the formation of genomic rearrangements like the TMPRSS2-ERG fusion oncogene in prostate cancer (2, 7, 8). It may be possible to exploit these hormone-induced DSB for cancer therapy by using various strategies to overwhelm the cancer cell with these transcription-associated DSB. Such strategies may find particular utility in cancers, like prostate cancer, that often show low proliferation rates, where other chemotherapeutic strategies that target rapidly proliferating cells have had limited success.

(1) Additional Authors: Michael C. Haffner, William G. Nelson

(2) Haffner MC et al., Nat Genet 2010; 42:668

(3) Ju BG et al., Science 2006; 312:1798

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Sources of Research Support: NIH Grant CA58236; Department of Defense Congressionally Directed Medical Research Program's Prostate Cancer Research Program Grant PC073533/W81XH-08-1-0049; The Prostate Cancer Foundation; Patrick C. Walsh Prostate Cancer Research Fund/Dr. and Mrs. Peter S. Bing Scholarship awarded to SY.

Nothing to Disclose: SY

Pub # S38-2

Session Information SYMPOSIUM SESSION: TRANSLATIONAL - Putting on the Brakes/Breaks in Prostate Cancer (3:45 PM - 5:15 PM)

Title Transcription, Growth & Translocation: A Synchrony of Movement, DNA Repeats & Non-Coding RNAs

Author String MG Rosenfeld
University of California, San Diego, La Jolla, CA

Body Our research focus has largely centered on uncovering new molecular strategies responsible for orchestrating and integrating programs of genome-wide transcriptional responses in development and in response to ligands, in the three dimensional space of the nucleus. Our goal is to ultimately develop new approaches to modulate transcriptional response in health and disease. Powerful new technologies, including next-generation sequencing, have ushered in a vastly altered conceptual landscape with respect to the transcriptional strategies that underlie both development and disease in all organ systems, including the cardiovascular system. Thus the roles of non-coding RNAs, DNA repeats formerly considered as [ldquo]junk DNA[rdquo] and regulated nuclear architecture have recently emerged as an important aspect of regulated programs of gene expression. The nuclear receptor (NR) superfamily of transcriptional regulators plays a central role in developmental homeostasis and diseases processes, and has been extensively studied as a model to identify the molecular mechanism for precise spatial and temporal control of gene expressions. Additional regulatory and sensor strategies have altered our views about mechanisms of gene regulation and disease. While the role of ligand-mediated nuclear receptors in mediating coactivator/corepressor exchange is well established, nuclear motor-dependent regulation of chromosomal organization in the three-dimensional space of the nucleus to diverse signaling events is emerging as a major parallel strategy to achieve integrated transcriptional responses revealing a key role for the modulation of nuclear architecture in orchestrating regulated gene expression programs in the mammalian nucleus, using previously unsuspected classes of sensor molecules. These events will be discussed with respect to tumor translocation events in prostate cancer.

Disclosure Incomplete: MGR

Pub # S38-3

Session Information SYMPOSIUM SESSION: TRANSLATIONAL - Putting on the Brakes/Breaks in Prostate Cancer (3:45 PM - 5:15 PM)

Title Developing Inhibitors to the N-Terminal Domain of the Androgen Receptor

Author String MD Sadar
British Columbia Cancer Agency, Vancouver, Canada

Body Androgen ablation therapy (castration) remains the gold standard for the treatment of advanced prostate cancer, but unfortunately, it is not curative and eventually the disease will return as lethal castration-resistant prostate cancer (CRPC). There is evidence supporting the concept that development of CRPC is causally related to continued transactivation of androgen receptor (AR). Suspected mechanisms for continued AR activity in spite of castrate levels of androgen include: amplification or overexpression of AR and/or coactivators; gain-of-function mutations allowing AR to be activated by steroids or antiandrogens; ligand-independent activation by growth factors, cytokines, or kinases; intracrine signaling by increased intratumoral androgens; and/or expression of constitutively active splice variants of AR that lack the C-terminal ligand-binding domain (LBD). All current therapies that target the AR are dependent on the presence of its LBD. However, it is the N-terminal domain (NTD) of the AR that is the [ldquo]Achilles Heel[rdquo] of AR activity. Activation function-1 (AF-1) in the NTD is essential for AR activity regardless of androgen. Our efforts have been focused upon developing drugs to the AR NTD and have yielded EPI-001 a small molecule, sintokamide peptides, and decoys to the AR NTD. Of these EPI-001 is the best characterized and blocked transactivation of the NTD. EPI-001 was specific for inhibition of AR without attenuating transcriptional activities of related steroid receptors. EPI-001 interacted with the AF-1 region, inhibited protein-protein interactions with AR, and reduced AR interaction with androgen-response elements on target genes. Importantly, EPI-001 blocked androgen-induced proliferation and caused cytoreduction of CRPC in xenografts dependent on AR for growth and survival without causing toxicity.

Sources of Research Support: Canadian Institutes of Health Research MOP89902 and the National Cancer Institutes 2R01 CA105304.

Nothing to Disclose: MDS

Pub # S39-1

Session Information SYMPOSIUM SESSION: CLINICAL - Endocrine Neoplasias from Top to Bottom (3:45 PM - 5:15 PM)

Title Mechanisms of Parathyroid Cancer from Inherited Syndromes

Author String WF Simonds
National Institute of Diabetes & Digestive & Kidney Diseases/National Institutes of Health, Bethesda, MD

Body Parathyroid cancer (PC), typically presenting with metabolic disturbances characteristic of severe primary hyperparathyroidism (HPT), is an uncommon endocrine malignancy with a high morbidity and patient death in advanced cases frequently resulting from intractable hypercalcemia. PC accounts for less than 1 % of cases of sporadic HPT, and is similarly rare in the context of familial endocrine tumor syndromes that include HPT such as multiple endocrine neoplasia type 1 and type 2A. In contrast, some 15% of HPT in the hyperparathyroidism-jaw tumor syndrome (HPT-JT) is due to PC. Genetic linkage analysis of affected members from HPT-JT kindreds showed that susceptibility results from germline inactivation of the *CDC73/HRPT2* gene on chromosome 1q25, encoding the putative tumor-suppressor protein parafibromin. Both alleles of the *CDC73/HRPT2* gene are inactivated in the majority of parathyroid cancers including apparently sporadic cases, in which some 25% carry germline *CDC73/HRPT2* mutation. Parafibromin is a protein of 531 amino acids localized primarily to the nucleus, by virtue of a bipartite nuclear localization signal, and further targeted to the nucleolus by additional signals. Like its homologs Cdc73p in budding yeast and Hyrax in *Drosophila*, human parafibromin forms part of a polymerase-associated factor 1 (PAF1) transcriptional regulatory complex. Besides parafibromin, the human PAF1 complex contains the proteins hPaf1, hCtr9, hLeo1, and WDR61 (hSki8). Parafibromin and the PAF1 complex promote chromatin modifications associated with transcriptional activation, such as Set1-like histone methyltransferase complex-mediated methylation of histone H3 on lysine 4 and FACT heterodimer-associated monoubiquitination of histone H2B on lysine 120. Furthermore as part of the PAF1 complex, parafibromin binds to the C-terminal domain of RNA polymerase II, and is involved in transcriptional initiation and elongation. Parafibromin binds directly to β -catenin as part of the PAF1 complex and promotes Wnt signalling. Interference with the expression of endogenous parafibromin inhibits basal and DNA damage-induced apoptosis, stimulates cell proliferation, and increases levels of the *c-myc* proto-oncogene product. The critical molecular pathways, by which loss of parafibromin function promotes the malignant transformation of parathyroid cells, either through disruption of PAF1 complex activity or by alternative mechanisms, remain to be elucidated.

Sources of Research Support: Intramural Research Program of the National Institute of Diabetes and Digestive and Kidney Diseases.

Nothing to Disclose: WFS

Pub # S39-2

Session Information SYMPOSIUM SESSION: CLINICAL - Endocrine Neoplasias from Top to Bottom (3:45 PM - 5:15 PM)

Title Increase in Testicular Germ Cell Cancer in Young Men: Origin from Dysgenesis of Fetal Testis

Author String NE Skakkebaek
University of Copenhagen, Copenhagen, Denmark

Body It has become evident that an increase in TGCC (seminoma, embryonal carcinoma, teratocarcinoma, yolk sac carcinoma and choriocarcinoma) first noticed in Denmark in the 1940s, is a worldwide phenomenon, particularly among Caucasians. However, noteworthy differences in incidences exist, even between neighbouring countries. Men living in the twin cities Copenhagen and Malm[ouml], connected by a bridge, are at significantly different risk of developing TGCC. Men at the Swedish side have much lower risk than the Danes. And men living in Finland have even lower risk than the Swedes. Several recent epidemiological studies, which have been based on these conspicuous differences are in agreement with a hypothesis of fetal origin of TGCC and a major impact of environment. Young Danes and Finns migrating to Sweden keep their high and low risk of TGCC, respectively. However, the next generations adapt to the intermediate risk typically found in Sweden. All subtypes of TGCC are derived from a carcinoma in situ (CIS) precursor. The CIS cell share properties with primordial germ cells and is probably derived from fetal gonocytes, which failed to differentiate into spermatogonia. Like normal fetal gonocytes the CIS cells express several markers of pluripotency, incl. Oct4, C-Kit, NANOG and AP2gamma. However, little is known about the fetal factors, which may inhibit differentiation of gonocytes resulting in CIS. The sharp increase in the incidence of TGCC over a short period of time and several immigrant studies shows that environmental factors are important, although familial TGCC shows that genetic aspects also operate in some cases. Recent GWAS studies have pointed towards several candidate genes, incl. KITLG. Importantly a high TGCC - rate in a population may be a proxy for other reproductive disorders in the same society, as there is evidence that populations with high risk of TGCC is also having high frequency of cryptorchidism, poor semen quality and hypospadias. We are now testing the hypothesis of fetal origin of TGCC and other adult testicular problems, which share a common etiology and may be different expressions of a testicular dysgenesis syndrome (TDS). Among the causative candidate factors are lifestyle exposures and endocrine disrupting chemicals, which may be particularly harmful for development of the fetal testis. If the fetal hypothesis is confirmed, prevention of TGCC should focus on women before and during reproductive ages.

Nothing to Disclose: NES

Pub # S39-3

Session Information SYMPOSIUM SESSION: CLINICAL - Endocrine Neoplasias from Top to Bottom (3:45 PM - 5:15 PM)

Title Highly Enriched Pluripotent Human Ovarian Cancer Cells Are Inhibited by Mullerian Inhibiting Substance

Author String PK Donahoe
Massachusetts General Hospital, Boston, MA

Body Tumor initiating cells are thought to be capable of unlimited self-renewal and differentiation and to confer characteristics of tumorigenicity, recurrence, metastasis, and drug resistance. We have identified in human ovarian cancer cell lines, a population (Epcam+/CD24+/CD44+; 3+) with stem cell characteristics as defined by increased colony formation and migration in vitro and early tumor formation in vivo. The 3+ cell population was further enriched with stem cell characteristics by negative selection for Ecadherin (3+Ecad-). Further testing showed that the 3+Ecad- cells were paradoxically stimulated by chemotherapeutic agents, but maintained sensitivity to Müllerian Inhibiting Substance (MIS) and an MIS mimetic, SP600125. In addition to increased colony formation in vitro and early tumor formation in vivo, the 3+Ecad cells were pluripotent as reflected by expression of Lin28, which is normally expressed in ovarian surface epithelium but is progressively upregulated in ovarian tumors of transgenic mice in which the MIS type II receptor (Misr2) is inactivated. MIS, a potent tumor suppressor, activates SMAD1/5/8 signaling through its type II receptor (Misr2), which inversely correlates with Lin28 expression. The 3+Ecad- population and the molecular mechanisms contributing to stem/progenitor phenotypes may offer novel therapeutic targets for ovarian cancer. As a result of our findings, we recommend that the stem/progenitor population of each patient's ovarian cancer should be separated and tested, prior to treatment, for drug sensitivity to chemotherapeutic agents and to biologics such as MIS.

Co-authors: Katia Meirelles^{1, 4}, Leo Andrew Benedict^{1, 4}, Fengsheng Chen^{1, 4}, David Dombkowski^{2, 4}, Kai Lu^{1,4}, Frederic I. Preffer^{2,4}, Jose Teixeira^{3,4}, David T. MacLaughlin^{1, 4}, and Xiaolong Wei^{1, 4,*}
1) Pediatric Surgical Research Laboratories, 2)Flow Cytometry Laboratory, 3) Vincent Center for Reproductive Biology, 4) Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114

Sources of Research Support: National Cancer Institute, National Institutes of Health.

Nothing to Disclose: PKD

Pub # S40-1

Session Information SYMPOSIUM SESSION: CLINICAL - Nonpharmacological Management of Osteoporosis (3:45 PM - 5:15 PM)

Title Optimizing Calcium & Vitamin D Intake for Prevention of Bone Loss

Author String JA Eisman
Garvan Institute of Medical Research, Sydney, Australia

Body Osteoporosis and associated fragility fractures have a major clinical impact, particularly in aging societies. Osteoporotic fractures, low bone density (BMD) and bone loss per se are all independently associated with mortality risk. Currently approved therapies have been shown unequivocally to reduce the risk of further fractures and possibly mortality risk. Virtually all of these effective therapies have been evaluated in people with adequate calcium intake and with vitamin D levels. It is not clear that these agents will work as effectively without optimized calcium & vitamin D status.

Adequate calcium intake and vitamin D levels are considered to be important complements to weight-bearing exercise to promote bone health and minimize bone loss. In the NHANES studies, while bone mineral density was significantly associated with measured serum vitamin D levels, there was little or no association with estimated calcium intake.

Studies evaluating the role of calcium and vitamin D in maintenance of bone density have reported a modest protective effect, but it is unclear whether these translate to reduced fracture risk. Two major meta-analyses have reported either a modest or no protective effect.

The safety of calcium supplementation has been challenged by a recent study and meta-analyses suggesting that it may increase risk of cardiovascular events. A RCT of annual high-dose vitamin D found increased falls and fractures. There were no baseline data on starting vitamin D status.

These possible adverse effects, combined with insufficient RCT data to support firm evidence-based conclusions of fracture risk reduction, have challenged the centrality of such interventions. Addressing conflicting studies requires judgement and opinion.

It appears prudent to suggest that women (and probably men) should have calcium intakes of the order of 1000 mg daily. Advocating adequate vitamin D levels also seems prudent; but what constitutes 'adequate' is being reevaluated. Serum values of 25-hydroxyvitamin D of 20-30ng/ml (50-75 nmol/L) seem reasonable and likely safe. Given the seasonal changes in vitamin D production related to sunlight exposure, a borderline value at the end of summer is probably more important than a similarly borderline value at the end of winter. These suggestions are opinion-based and are largely targeted to prevention. It is clear that alone these could not be considered adequate therapy for established osteoporosis with prior low trauma fractures.

Nothing to Disclose: JAE

Pub #	S40-2
Session Information	SYMPOSIUM SESSION: CLINICAL - Nonpharmacological Management of Osteoporosis (3:45 PM - 5:15 PM)
Title	Exercise & Fall Prevention as Osteoporosis Management Strategies
Author String	WS Gozansky Kaiser Permanente Colorado, Arvada, CO
Body	Nothing to Disclose: WSG

Pub #	S40-3
Session Information	SYMPOSIUM SESSION: CLINICAL - Nonpharmacological Management of Osteoporosis (3:45 PM - 5:15 PM)
Title	Bad Habits & Bone: Alcohol, Tobacco & Caffeine
Author String	SM Jan De Beur Johns Hopkins University School of Medicine, Baltimore, MD
Body	Nothing to Disclose: SMJDB

Pub #	S41-1
Session Information	SYMPOSIUM SESSION: CLINICAL - Therapeutic Interventions for Childhood Obesity: What Should We Expect? (3:45 PM - 5:15 PM)
Title	Childhood Obesity & Premature Mortality: Targeting Co-Morbidities for Therapeutic Interventions
Author String	S Caprio Yale University, New Haven, CT
Body	Nothing to Disclose: SC

Pub # S41-2

Session Information SYMPOSIUM SESSION: CLINICAL - Therapeutic Interventions for Childhood Obesity: What Should We Expect? (3:45 PM - 5:15 PM)

Title Present & Future of Pharmacological Interventions in Pediatric Obesity: Who, When & Which?

Author String J-P Chanoine
British Columbia Children's Hospital, Vancouver, Canada

Body Family-based interventions that include improved diet, decreased inactivity and behaviour change represent the most common approaches for the treatment of obesity in youth. Although they can reduce or delay metabolic complications, overall outcomes remain unsatisfactory and short-lived. Bariatric surgery in contrast is very effective in promoting weight loss, but access is difficult and long-term effects in adolescents remain unclear. Pharmacotherapy has the potential of greatly improving the outcome of weight loss interventions in pediatrics but so far has shown modest results.

Present: Orlistat is an intestinal lipase inhibitor that prevents absorption of up to 1/3 of dietary fat and the only pharmacological agent approved by the FDA for children >12 years. Given at 120 mg TID in addition to a lifestyle intervention, orlistat caused an additional 0.86 kg/m² decrease in BMI after 12 months compared to placebo, a result fully explained by changes in fat mass. Gastrointestinal side effects were more common in orlistat-treated subjects (1). Early weight loss with orlistat predicted a greater BMI decrease at 12 months: BMI decreased by 3.7 kg/m² in subjects who lost >5% body weight after 3 months but increased by 0.1 kg/m² in those who lost <5% body weight (2). Metformin is an anti-diabetic agent that improves insulin sensitivity and is being investigated for weight loss in obese, hyperinsulinemic adolescents. It is presently not FDA-approved for this indication. A meta-analysis of 5 randomized trials of 6 months' duration showed that, compared to placebo, metformin decreases BMI by an additional 1.42 kg/m² compared to placebo-treated subjects and improves insulin sensitivity (3). Similar results (1.1 kg/m²) were recently observed after treatment for 11 months (4).

Future: In order for pharmacological options to become more effective, we need to: 1. understand the adolescent-specific characteristics of physiological circuits involved in energy balance; 2. develop safe, well-tolerated and effective drugs that address specific physiological systems (5); 3. promote targeted pharmacotherapy that addresses specific individual characteristics; and 4. design clinical trials where obese adolescents are enrolled according to well-defined inclusion and exclusion criteria (type of eating, level of activity, behaviour, metabolic profile, etc.) and followed for more 12 months or more.

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(4) Glaser Ped Res Network, Arch Pediatr Adolesc Med. 2010;164: 116
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Nothing to Disclose: J-PC

Pub # S41-3

Session Information SYMPOSIUM SESSION: CLINICAL - Therapeutic Interventions for Childhood Obesity: What Should We Expect? (3:45 PM - 5:15 PM)

Title Surgical Interventions for Obesity in Adolescents & Young Adults

Author String EP Nadler
George Washington University School of Medical & Health Sciences, Washington, DC

Body Obesity has been clearly identified as one of the most important public health concerns in both children and adolescents. Unfortunately, even the most comprehensive and aggressive multi-disciplinary weight management programs have shown only modest results once the adolescent has become morbidly obese. Thus there has been increasing enthusiasm for bariatric surgery to treat morbid obesity in adolescents who are suffering from obesity-related comorbid conditions. Until recently this enthusiasm has focused mainly on the laparoscopic gastric bypass (LGB). It has been previously demonstrated that the nutritional and behavioral modifications associated with bariatric surgery for adolescents in general, and the LGB specifically, are associated with significant weight loss, as well as correction of comorbid conditions and improved self-image and socialization. However, due to the relatively high morbidity and mortality associated with the LGB some experts have recommended stricter criteria than the NIH consensus guidelines for adults to perform the LGB in morbidly obese adolescents. However this strategy may severely limit access for obese adolescents to what may be their only pathway to sustained weight loss and resolution of comorbidities. Therefore a need has arisen to find alternative options for morbidly obese adolescents that could result in similar weight loss and more importantly similar resolution of comorbidities, without the significant risk associated with the LGB. The high morbidity and mortality associated with the LGB has led us to pursue utilizing the laparoscopic adjustable gastric band (LAGB) for morbidly obese adolescents. Since its FDA approval for patients older than 18 years in 2001, the LAGB has been shown to have a lower complication rate and a much milder complication profile than the other surgical options. These traits make the LAGB an attractive choice for the adolescent population. In this presentation, we will discuss the results from prospective studies using both LGB and LAGB. We are now beginning to explore a second restrictive only procedure known as the laparoscopic sleeve gastrectomy (LSG). Early results using this newer procedure in morbidly obese adolescents will also be shared and discussed.

Nothing to Disclose: EPN

Pub #	NS7-1
Session Information	ENDOCRINE NURSES SYMPOSIUM: CLINICAL - Endocrine Cancer: Medullary Thyroid Cancer (4:00 PM - 5:00 PM)
Title	Endocrine Cancer: Medullary Thyroid Cancer
Author String	RC Smallridge Mayo Clinic Jacksonville, Jacksonville, FL
Body	Disclosure Incomplete: RCS

Pub # L4-1

Session Information PLENARY: TRANSLATIONAL - Tissue-Specific Regulation of Thyroid Hormone Action (5:30 PM - 6:30 PM)

Title Regulation of Tissue-Specific Thyroid Hormone Action

Author String AC Bianco
University of Miami Miller School of Medicine, Miami, FL

Body In all vertebrates, the iodothyronine deiodinases are critical for the biological effects mediated by thyroid hormone as they initiate or terminate thyroid hormone action. The activating deiodinase (D2) and the inactivating deiodinase (D3) control thyroid hormone action at the cellular level, increasing or decreasing thyroid hormone signaling in a tissue- and temporal-specific fashion, independently of changes in thyroid hormone serum concentrations. From a broad perspective, deiodination can be seen as an example of a paradigm in which hormones are activated or inactivated in a controlled fashion in specific extraglandular tissues, in a role analogous to that of 5 α -reductase and P450 aromatase in sex steroid metabolism and of 11 β -hydroxysteroid dehydrogenase in glucocorticoid metabolism. These deiodinase-based mechanisms are indeed physiologically relevant during development and after birth, throughout health and disease. In the embryo, there are rapid reciprocal changes in D2 and D3 expression, modulating thyroid hormone signaling during critical tissue-specific developmental check-points such as in the brown adipose tissue. This also takes place in the hypothalamus and pituitary gland, ultimately regulating TSH secretion. After birth, thyroid hormone activation through deiodination (via D2) plays a role in energy homeostasis, accelerating T3 production to increase energy expenditure during cold exposure or feeding a hypercaloric diet. On the other hand, in tissue injury and disease states there is induction of the inactivation pathway (via D3) that can be tissue-specific or widespread, in which case it underlies the development of the [ldquo]euthyroid sick syndrome[rdquo]. In all of these setting, the expression of the deiodinases is modulated transcriptionally and post-transcriptionally by a wide variety of endogenous signaling molecules such as sonic hedgehog, NF-kB, growth factors, bile acids, HIF-1a, as well as a growing number of xenobiotic substances and chemical chaperones. Thus, deiodinases seem to play a much broader role than once thought, with great ramifications for the control of thyroid hormone signaling during vertebrate development, as well as injury response, tissue repair, hypothalamic function, and energy homeostasis in adults. The therapeutic potential is obvious: if the D2 and D3 pathways can be harnessed pharmacologically, the resulting control of thyroid hormone action on a tissue-specific fashion may prove to be particularly useful.

Sources of Research Support: NIDDK.

Nothing to Disclose: ACB

Pub # L4-2

Session Information PLENARY: TRANSLATIONAL - Bone Morphogenetic Proteins in the Musculoskeletal System (5:30 PM - 6:30 PM)

Title Bone Morphogenetic Proteins in the Musculoskeletal System

Author String V Rosen
Harvard School of Dental Medicine, Boston, MA

Body BMPs are locally acting signaling molecules that were identified over 20 years ago based on their ability to initiate ectopic bone formation in adult animals. Subsequent gain and loss of function studies in mice demonstrated that BMPs play an essential role in the development of nearly all vertebrate organs, including those that make up the musculoskeletal system. Musculoskeletal cells remain BMP targets throughout life when BMP signaling affects both the growth and repair potentials of bone, tendon, ligament, muscle, and articular cartilage. In these tissues, BMP signaling is part of a complex signaling cascade that orchestrates the interactions of Wnts, PTH, and IGF signaling to maintain musculoskeletal tissue homeostasis.

Nothing to Disclose: VR

Pub # M27

Session Information MEET-THE-PROFESSOR: CLINICAL - Management Issues in Adult Growth Hormone Replacement (5:30 PM - 6:15 PM)

Title Management Issues in Adult Growth Hormone Replacement

Author String P Chanson
Hospital Bicetre, Le Kremlin-Bicetre, France

Body Session supported by: Novo Nordisk Inc. & Pfizer, Inc.

Disclosures: PC: Advisory Group Member, Eli Lilly & Company; Clinical Researcher, Ipsen, Merck & Co., Novartis Pharmaceuticals, Novo Nordisk, Pfizer, Inc.

Pub # CMF5-1
Session Information CASE MANAGEMENT FORUM: CLINICAL - Gauging Response to Osteoporosis Therapy (5:30 PM - 6:15 PM)
Title Gauging Response to Osteoporosis Therapy
Author String CB Niewoehner
Minneapolis Veterans Affairs Medical Center, Minneapolis, MN
Body Session supported by: Amgen

Nothing to Disclose: CBN

Pub # CMF5-2
Session Information CASE MANAGEMENT FORUM: CLINICAL - Gauging Response to Osteoporosis Therapy (5:30 PM - 6:15 PM)
Title Gauging Response to Osteoporosis Therapy
Author String CJ Rosen
Maine Medical Center Research Institute, Scarborough, ME
Body Session supported by: Amgen

Nothing to Disclose: CJR

Pub #	M22
Session Information	MEET-THE-PROFESSOR: CLINICAL - Contraception across the Span of Child-Bearing Years (5:30 PM - 6:15 PM)
Title	Contraception across the Span of Child-Bearing Years
Author String	GM Christman University of Michigan, Ann Arbor, MI
Body	Nothing to Disclose: GMC

Pub #	M23
Session Information	MEET-THE-PROFESSOR: CLINICAL - Controversies in Cushing Syndrome (5:30 PM - 6:15 PM)
Title	Controversies in Cushing Syndrome
Author String	JDC Newell-Price University of Sheffield, Sheffield, UK
Body	Session supported by: Corcept Therapeutics Disclosure Incomplete: JDCN-P

Pub #	M24
Session Information	MEET-THE-PROFESSOR: CLINICAL - Disorders of Sex Development in Children (5:30 PM - 6:15 PM)
Title	Disorders of Sex Development in Children
Author String	JC Achermann University College of London Institute of Child Health, London, UK
Body	Nothing to Disclose: JCA

Pub # M25
Session Information MEET-THE-PROFESSOR: CLINICAL - Insulin Pumps (5:30 PM - 6:15 PM)
Title Insulin Pumps
Author String AL McCall
University of Virginia Health System, Charlottesville, VA
Body Session supported by: Lilly USA, LLC

Nothing to Disclose: ALM

Pub #	M26
Session Information	MEET-THE-PROFESSOR: CLINICAL - Issues in the Diagnosis & Treatment of Klinefelter Syndrome (5:30 PM - 6:15 PM)
Title	Issues in the Diagnosis & Treatment of Klinefelter Syndrome
Author String	AS Dobs Johns Hopkins University School of Medicine, Baltimore, MD
Body	Nothing to Disclose: ASD

Pub #	M28
Session Information	MEET-THE-PROFESSOR: CLINICAL - Treatment of Severe Thyrotoxicosis (5:30 PM - 6:15 PM)
Title	Treatment of Severe Thyrotoxicosis
Author String	MT McDermott University of Colorado Hospital, Denver, CO
Body	Nothing to Disclose: MTM

Pub #

Session Information

CMES SYMPOSIA: Individualizing Diabetes Treatment for Improved Glycemic Control and Management of Comorbidities (6:30 PM - 9:30 PM)

Title

Individualizing Diabetes Treatment for Improved Glycemic Control and Management of Comorbidities

Author String

Body

This activity is supported by an independent educational grant from Boehringer Ingelheim Pharmaceuticals, Inc. which was made possible, in part, through a collaboration with Eli Lilly and Company

Pub #

Session Information

CONFERENCE EVENT: ENDO Trivia Cup Challenge (6:45 PM - 8:30 PM)

Title

ENDO Trivia Cup Challenge

Author String

Body

Pub #

Session Information

CONFERENCE EVENT: Minority Mentoring & Poster Reception (7:00 PM - 9:30 PM)

Title

Minority Mentoring & Poster Reception

Author String

Body

Pub #

Session Information

CMES SYMPOSIA: Medical Management of Prolactinomas, Cushing's Disease and Acromegaly: A Case-Based Approach (6:00 AM - 8:00 AM)

Title

Medical Management of Prolactinomas, Cushing's Disease and Acromegaly: A Case-Based Approach

Author String

Body

Novartis Pharmaceuticals Corporation

Pub # L5-1

Session Information PLENARY: TRANSLATIONAL - Edwin B Astwood Award Lecture: Protein Folding Homeostasis & Metabolic Regulation (8:00 AM - 9:15 AM)

Title Edwin B Astwood Award Lecture: Protein Folding Homeostasis & Metabolic Regulation

Author String D Ron
University of Cambridge, Cambridge, UK

Body A significant portion of intercellular communication depends on protein secretion and interpretation of extracellular signals by transmembrane receptors. Proteins of both classes fold into their functional state in the lumen of the endoplasmic reticulum (ER), a process that is assisted by a dedicated machinery of ER-associated chaperones, enzymes and other components. The magnitude of this apparatus is matched to the load of unfolded proteins confronting the ER by a cell autonomous signalling pathway referred to as the Unfolded Protein Response. The biological significance of the unfolded protein stress (ER stress) that the UPR counteracts is attested to by the deleterious consequences of mutations in signalling components of the UPR or in elements of the ER protein folding machinery. The study of extreme cases of failure of protein folding homeostasis in the secretory pathway has revealed its impact on endocrine signalling, with the endocrine pancreas manifesting special susceptibility. Of potentially greater clinical significance are hints that long term exposure to low levels of ER stress may contribute to attrition in secretory function, which may play a role in common forms of diabetes mellitus.

Intriguingly, factors that influence protein folding homeostasis in the ER also have an evolutionarily-conserved dialectic relationship with intermediary metabolism and with its hormonal control. This is reflected in the influence of nutrient availability on protein folding in the ER and in an overlap between the readouts of the mammalian UPR and that of other pathways detect nutrient sufficiency.

In this talk I will attempt to summarize the aforementioned relationships and discuss their physiological and pathophysiological significance

Sources of Research Support: NIDDK47119 and the Wellcome Trust.

Nothing to Disclose: DR

Pub # L5-2

Session Information PLENARY: TRANSLATIONAL - Glucocorticoids in Fetal Programming (8:00 AM - 9:15 AM)

Title Glucocorticoids in Fetal Programming

Author String JR Seckl
University of Edinburgh, Edinburgh, UK

Body Epidemiological evidence suggests that an adverse fetal environment permanently programmes physiology leading to increased risks of cardiometabolic, neuroendocrine and psychiatric disorders in adulthood. We originally hypothesised that fetal glucocorticoid overexposure might explain this link. In rodents, prenatal stress, glucocorticoid exposure or inhibition/knockout of 11 β -hydroxysteroid dehydrogenase type 2 (11 β -HSD2), the feto-placental 'barrier' to maternal glucocorticoids, reduces birth weight and causes permanent hypertension, hyperglycaemia, increased hypothalamic-pituitary-adrenal (HPA) axis activity and anxiety-related behaviours in adult offspring. The phenotype persists into a second generation and transmits via male and female lines. This implies epigenetic mediation, a mechanism emerging for HPA axis programming. This also appears of potential clinical relevance. Thus, in non-human primates, exposure to glucocorticoids in the second half of gestation programmes cardiometabolic, HPA and behavioural parameters in offspring. In humans, placental 11 β -HSD2 activity correlates directly with birth weight and inversely with infant blood pressure. Moreover, low birth weight babies have higher plasma cortisol levels throughout adult life, indicating HPA programming. Indeed, maternal glucocorticoid therapy or ingestion of liquorice (which inhibits 11 β -HSD) alters offspring cognition, behaviour and HPA function. Stress has similar effects since pregnant women exposed to the 9.11.2001 atrocity and who developed PTSD 'transmit' neuroendocrine changes to their one-year old offspring, but confined to third trimester exposure. Furthermore, exposure to the Nazi Holocaust exerted permanent effects upon glucocorticoid levels and steroid metabolism, effects dependent upon the age at exposure. The second (un-exposed) generation also shows altered cortisol levels and metabolism. Overall, the data suggest that developmental exposure to excess glucocorticoids/stress programmes peripheral and CNS functions in adult life, predisposing to affective and other pathology, and these effects may impact on a subsequent generation.

Nothing to Disclose: JRS

Pub # CMF8-1
Session Information CASE MANAGEMENT FORUM: CLINICAL - Investigation & Management of Gynecomastia (8:30 AM - 9:15 AM)
Title Investigation & Management of Gynecomastia
Author String GD Braunstein
Cedars-Sinai Medical Center, Los Angeles, CA
Body Disclosures: GDB: Consultant, Abbott Laboratories, Esoterix (LabCorp); Principal Investigator, BioSante.

Pub #	CMF8-2
Session Information	CASE MANAGEMENT FORUM: CLINICAL - Investigation & Management of Gynecomastia (8:30 AM - 9:15 AM)
Title	Investigation & Management of Gynecomastia
Author String	BB Yeap University of Western Australia, Fremantle, Australia
Body	Nothing to Disclose: BBY

Pub #	M29
Session Information	MEET-THE-PROFESSOR: CLINICAL - Cases from the Metabolic Bone Clinic (8:30 AM - 9:15 AM)
Title	Cases from the Metabolic Bone Clinic
Author String	M Kleerekoper Wayne State University, Bloomfield Hills, MI
Body	Disclosures: MK: Speaker Bureau Member, Amgen, Eli Lilly & Company; Advisory Group Member, Roche Diagnostics, Ortho McNeil.

Pub # M30
Session Information MEET-THE-PROFESSOR: CLINICAL - Diagnostic Strategy for Post-Menopausal Androgen Excess (8:30 AM - 9:15 AM)
Title Diagnostic Strategy for Post-Menopausal Androgen Excess
Author String ME Wierman
Veterans Affairs Medical Center, Endocrine 111H, Denver, CO
Body Disclosure Incomplete: MEW

Pub #	M31
Session Information	MEET-THE-PROFESSOR: CLINICAL - Dopamine Agonists: When To Use, What To Choose & Safety Issues (8:30 AM - 9:15 AM)
Title	Dopamine Agonists: When To Use, What To Choose & Safety Issues
Author String	JA Romijn Academic Medical Center, Amsterdam, Netherlands
Body	Nothing to Disclose: JAR

Pub #	M32
Session Information	MEET-THE-PROFESSOR: CLINICAL - Management of Hormone Replacement in Addison Disease (8:30 AM - 9:15 AM)
Title	Management of Hormone Replacement in Addison Disease
Author String	BM Arafah Case Western Reserve University, Cleveland, OH
Body	Nothing to Disclose: BMA

Pub #	M33
Session Information	MEET-THE-PROFESSOR: CLINICAL - Management of Hypogonadotropic Hypogonadism (8:30 AM - 9:15 AM)
Title	Management of Hypogonadotropic Hypogonadism
Author String	AC Latronico Sao Paulo University, Sao Paulo, Brazil
Body	Nothing to Disclose: ACL

Pub # M34
Session Information MEET-THE-PROFESSOR: CLINICAL - Management of Idiopathic Short Stature (8:30 AM - 9:15 AM)
Title Management of Idiopathic Short Stature
Author String SD Chernausek
Oklahoma Health Sciences Center, Oklahoma City, OK
Body Session supported by: Lilly USA, LLC, Novo Nordisk Inc. & Pfizer, Inc.
Disclosure Incomplete: SDC

Pub # CMF6-1

Session Information CASE MANAGEMENT FORUM: CLINICAL - AACE vs ADA Guidelines for Diagnosis & Management of Type 2 Diabetes Mellitus (8:30 AM - 9:15 AM)

Title AACE vs ADA Guidelines for Diagnosis & Management of Type 2 Diabetes Mellitus

Author String JA Davidson
University of Texas Southwestern Medical Center, Dallas, TX

Body Session supported by: Amylin Pharmaceuticals, Inc., Lilly USA, LLC and Merck & Co., Inc.

Disclosure Incomplete: JAD

Pub # CMF6-2

Session Information CASE MANAGEMENT FORUM: CLINICAL - AACE vs ADA Guidelines for Diagnosis & Management of Type 2 Diabetes Mellitus (8:30 AM - 9:15 AM)

Title AACE vs ADA Guidelines for Diagnosis & Management of Type 2 Diabetes Mellitus

Author String DM Nathan
Massachusetts General Hospital/Harvard Medical School, West Newton, MA

Body Session supported by: Amylin Pharmaceuticals, Inc., Lilly USA, LLC and Merck & Co., Inc.

Nothing to Disclose: DMN

Pub #	CMF7-1
Session Information	CASE MANAGEMENT FORUM: CLINICAL - Diagnosis of Thyroid Cancer Using Ultrasound & Fine Needle Aspiration (8:30 AM - 9:15 AM)
Title	Diagnosis of Thyroid Cancer Using Ultrasound & Fine Needle Aspiration
Author String	B McIver Mayo Clinic, Rochester, MN
Body	Nothing to Disclose: BM

Pub #	CMF7-2
Session Information	CASE MANAGEMENT FORUM: CLINICAL - Diagnosis of Thyroid Cancer Using Ultrasound & Fine Needle Aspiration (8:30 AM - 9:15 AM)
Title	Diagnosis of Thyroid Cancer Using Ultrasound & Fine Needle Aspiration
Author String	RT Kloos Ohio State University, Columbus, OH
Body	Disclosures: RTK: Study Investigator, Veracyte, Inc.

Pub # S42-1

Session Information SYMPOSIUM SESSION: BASIC - Novel Aspects of Signaling through G Protein-Coupled Receptors (9:30 AM-11:00 AM)

Title Regulation of Parathyroid Hormone Receptor Signaling by Sodium-Hydrogen Exchange Regulatory Factors

Author String PA Friedman
University of Pittsburgh School of Medicine, Pittsburgh, PA

Body The parathyroid hormone receptor (PTHr) is a Family B G protein-coupled receptor that mediates the biological actions of both parathyroid hormone (PTH) and of the PTH-related protein. The PTHr is expressed primarily in bone and in kidney cells, where it regulates skeletal growth and turnover, and extracellular mineral-ion homeostasis, respectively. In some cells PTHr activation stimulates adenylyl cyclase, whereas in others it stimulates phospholipase C (PLC). In still other cell types such as osteoblasts and kidney tubule cells both signaling pathways may be activated. The origin of this cell-specific signaling pattern remained unclear until the remarkable discovery by Mahon and Segre in 2002 that the Na/H Exchange Regulatory Factor (NHERF), a PDZ adapter protein, present in some but not all cells expressing the PTHr, switches signaling between adenylyl cyclase and PLC. The Na/H exchanger regulatory factors form a family of adaptor proteins consisting of four members. NHERF1 and NHERF2 possess two non-identical, type 1 tandem PDZ domains, whereas NHERF3 and NHERF4 contain four. NHERF1 and NHERF2 have a carboxy-terminal ezrin-binding domain, which indirectly tethers these proteins to the actin cytoskeleton. Increasing evidence now supports the view that cytoplasmic adapter proteins affect the signaling and trafficking of many GPCRs, and thereby their biological behavior. NHERF1 importantly regulates ligand bias at the PTH1R, PTH1R desensitization, and trafficking of the PTH1R. NHERF1-null mice generated by homologous recombination of exon 1 exhibit profound hypophosphatemia. Likewise, humans harboring NHERF1 polymorphisms display a similar presentation with conspicuous renal phosphate wasting. Interestingly, these variants, which are located in the linker region between PDZ1 and PDZ2, or in PDZ2, do not interfere with PTH-stimulated cAMP accumulation when heterologously expressed. Thus, the disordered phosphate transport arises from an allosteric action of NHERF1 on PTHr binding or interference with a posttranslational modification. Work in progress suggests that the described NHERF1 polymorphisms stabilize a closed conformation of NHERF1 that is unable to form a functional ternary complex with the Npt2a phosphate transporter and ezrin. In this talk, emerging findings regarding the means by which NHERF proteins confer ligand- and cell-specific signaling and trafficking, and functioning of the PTHr will be described.

Sources of Research Support: NIH R01 DK054171, R01 DK069998.

Nothing to Disclose: PAF

Pub # S42-2

Session Information SYMPOSIUM SESSION: BASIC - Novel Aspects of Signaling through G Protein-Coupled Receptors (9:30 AM-11:00 AM)

Title Refining Efficacy: Exploiting Arrestin Pathway-Selectivity To Achieve Unique Ligand Efficacy In Vivo

Author String LM Luttrell
Medical University of South Carolina, Charleston, SC

Body Heptahelical G protein-coupled receptors (GPCRs) are the most diverse and therapeutically important family of hormone receptors. In addition to signaling through heterotrimeric G proteins, GPCRs couple to non-G protein effectors such as arrestins. Arrestins engage several catalytically active proteins and act as ligand-regulated scaffolds that recruit kinase, phosphatase, phosphodiesterase and ubiquitin ligase activity into GPCR-arrestin signalsomes. Biased GPCR agonists are orthosteric ligands that possess pathway-selective efficacy, activating or inhibiting only a subset of the signaling repertoire of their cognate receptors. Thus, biased agonists have the capacity to qualitatively change GPCR signaling. In vitro, D-Trp12,Tyr34-bPTH (7-34) (PTH- β Arr), a biased agonist for the type 1 parathyroid hormone receptor (PTH1R), antagonizes receptor-G protein coupling but activates arrestin-dependent signaling. In vivo, both PTH- β Arr and the conventional agonist PTH(1-34) stimulate anabolic bone formation. While both ligands raise indices of bone formation and increase trabecular bone mass, PTH- β Arr does not accelerate bone resorption, suggesting it does not promote osteoblast-osteoclast coupling. To understand how two PTH1R ligands with such different in vitro efficacy could elicit similar in vivo responses, we performed functional genomic analysis of bone from mice treated with vehicle, PTH- β Arr or PTH(1-34). Treatment of wild type mice with PTH- β Arr primarily affected gene clusters involved in cell cycle regulation, cell survival and migration. These responses were absent in β -arrestin2 null mice, identifying them as downstream targets of arrestin-mediated signaling. In contrast, PTH(1-34) primarily affected pathways classically associated with enhanced bone formation, including collagen synthesis and matrix mineralization. PTH(1-34) actions were less dependent on β -arrestin2 as might be expected of a ligand capable of G protein activation. Confirmatory in vitro experiments demonstrated that PTH- β Arr promotes osteoblast survival and stimulates migration, suggesting that its anabolic effects arise from selective expansion of the osteoblast pool rather than from increased rates of bone matrix synthesis and mineralization. These results illustrate the uniqueness of biased agonism in vivo and demonstrate that functional selectivity can be exploited to produce biological responses not obtainable using conventional agonist or antagonist ligands.

The presenting author gratefully acknowledges the contributions of the following equal co-authors:
Diane Gesty-Palmer, MD, PhD, Duke Univ Med Center
Stuart Maudsley, PhD, Nat Inst on Aging.

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Nothing to Disclose: LML

Pub # S42-3

Session Information SYMPOSIUM SESSION: BASIC - Novel Aspects of Signaling through G Protein-Coupled Receptors (9:30 AM-11:00 AM)

Title The Human Gonadotropin-Releasing Hormone Type 1 Receptor Is a Functional Intracellular G Protein-Coupled Receptor Expressed on the Nuclear Membrane

Author String AV Babwah
Children's Health Research Institute, London, Canada

Body The mammalian type I gonadotropin releasing hormone receptor (GnRH-R) is a structurally unique G protein-coupled receptor (GPCR) that lacks cytoplasmic tail sequences and displays inefficient plasma membrane expression (PME). Compared to its murine counterparts, the primate type I receptor is inefficiently folded and retained in the endoplasmic reticulum (ER) leading to a further reduction in PME. The decrease in PME and concomitant increase in intracellular localization of the mammalian GnRH-R led us to characterize the spatial distribution of the human and mouse GnRH receptors in two human cell lines, HEK 293 and HTR-8/SVneo. In both human cell lines we found the receptors were expressed in the cytoplasm and were associated with the ER and nuclear membrane. A molecular analysis of the receptor protein sequence led us to identify a putative monopartite nuclear localization sequence (NLS) in the first intracellular loop of GnRH-R. Surprisingly, however, neither the deletion of the NLS nor the addition of the *Xenopus* GnRH-R cytoplasmic tail sequences to the human receptor altered its spatial distribution. Finally, we demonstrate that GnRH treatment of nuclei isolated from HEK 293 cells expressing exogenous GnRH-R triggers a significant increase in the acetylation and phosphorylation of histone H3, thereby revealing that the nuclear-localized receptor is functional. Based on our findings, we conclude that the mammalian GnRH-R is an intracellular GPCR that is expressed on the nuclear membrane. This novel discovery causes us to reassess the signaling potential of this physiologically and clinically important receptor.

Nothing to Disclose: AVB

Pub # S47-1

Session Information SYMPOSIUM SESSION: TRANSLATIONAL - Thyroid-Stimulating Hormone in Thyroid Cancer Development & Progression: From Mouse to Man (9:30 AM - 11:00 AM)

Title Role of Thyroid-Stimulating Hormone in Mouse Models of Thyroid Cancer

Author String S-y Cheng
National Cancer Institute/National Institutes of Health, Bethesda, MD

Body Thyroid stimulating hormone (TSH) is the major stimulator of thyrocyte proliferation, but its role in thyroid carcinogenesis remains unclear. To study the role of TSH in thyroid carcinogenesis *in vivo*, we used a mouse model of follicular thyroid cancer (ThrbPV/PV mice). This mouse model has unique characteristics that make it well suited for use in understanding the role of TSH in cancer development. The ThrbPV/PV mouse harbors a knockin dominantly negative mutation of the thyroid hormone β receptor gene (Thrb), denoted as PV. As ThrbPV/PV mice age, they spontaneously develop follicular thyroid carcinoma (FTC) with pathological progression, cytohistological features, and metastasis patterns and frequency similar to those of human FTC. ThrbPV/PV mice exhibit increased serum thyroid hormone and highly elevated TSH. To eliminate the TSH-growth stimulating effect, ThrbPV/PV mice were crossed with TSH receptor gene knockout (Tshr^{-/-}) mice. Wild-type siblings of ThrbPV/PV mice were treated with an anti-thyroid agent, propylthiouracil (PTU), to elevate serum TSH for evaluating long-term TSH effect (WT-PTU mice). Thyroids from ThrbPV/PVTshr^{-/-} mice showed impaired growth, but exhibited no occurrence of FTC. Both WT-PTU and ThrbPV/PV mice displayed enlarged thyroids, but only ThrbPV/PV mice developed metastatic FTC. Molecular analyses indicate that PV acted, via multiple mechanisms, to activate the integrin-Src-focal adhesion kinase (FAK)-p38 MAPK-matrix metalloproteinase-9 pathway and to affect actin-cytoskeleton restructuring to increase tumor cell motility, migration, and invasion. Thus, growth stimulated by TSH is prerequisite, but not sufficient for metastatic thyroid cancer to occur. Additional genetic alterations (such as PV), destined to alter focal adhesion and migration capacities, are required to empower hyperplastic follicular cells to invade and to metastasize. These *in vivo* findings provide new insights into the role of TSH in carcinogenesis of the human thyroid.

Nothing to Disclose: S-YC

Pub # S47-2

Session Information SYMPOSIUM SESSION: TRANSLATIONAL - Thyroid-Stimulating Hormone in Thyroid Cancer Development & Progression: From Mouse to Man (9:30 AM - 11:00 AM)

Title Role of Thyroid-Stimulating Hormone in Risk of Cancer in Thyroid Nodules

Author String MR Haymart
University of Michigan, Ann Arbor, MI

Body Within the past five years there has been a growing body of literature describing an association between thyroid stimulating hormone (TSH) level and thyroid cancer incidence. A higher TSH, even within normal range, has been associated with a greater likelihood of thyroid cancer in thyroid nodule patients. This relationship between TSH and thyroid cancer has been demonstrated in several different patient populations. In addition, more recent studies have found a relationship between TSH level and more aggressive disease. This presentation will address the recent studies on TSH and risk of cancer in thyroid nodules and the potential clinical implications of these studies.

Nothing to Disclose: MRH

Pub # S47-3

Session Information SYMPOSIUM SESSION: TRANSLATIONAL - Thyroid-Stimulating Hormone in Thyroid Cancer Development & Progression: From Mouse to Man (9:30 AM - 11:00 AM)

Title Thyroid-Stimulating Hormone Suppression in Thyroid Cancer

Author String SL Lee
Boston Medical Center, Boston, MA

Body Thyroid hormone therapy is a one of the three cornerstones for the treatment of differentiated thyroid cancer: surgery, radioactive iodine ablation/therapy and thyroid hormone suppression of TSH. It is generally believed that thyroid hormone therapy suppresses tumor growth via the negative feedback inhibition of pituitary TSH secretion. But where did this concept develop? The earliest observation may be by Reinhold and Bruns in the 1890's who reported the effectiveness of thyroid extract to shrink goiter. It is believed that the success of shrinking hypothyroid and iodine deficiency goiters with thyroid hormone extract permitted trial in patients with thyroid cancer as reported by Dunhill in the in1930's. TSH is a trophic factor for thyroid follicular cells to increase metabolic rate, glucose utilization, cell hypertrophy and cell division. Bench research confirms tha TSH stimulates primary thyroid cell cultures to increase cyclic AMP, metabolic rate, thyroglobulin synthesis, and iodine uptake. Investigators using cDNA microarray containing 2400 genes have shown that more than 100 genes were activated in follicular cells in culture including those involved with adenylate and guanylate cyclases, carbohydrate and lipid metabolism and cell proliferation. But TSH receptor activation may not induce tumorigenesis as suggested by the uncommon occurrence of malignancy seen in toxic adenomas which frequently contain unregulated TSH receptor mutations and in patients with immunoglobulin activation of the TSH receptor in Graves' disease. Although it has been assumed that TSH suppression by thyroid hormone may produce tumor progression and/or recurrence but there are no prospectively designed trials showing that undetectable TSH is more effective than less suppressed levels of TSH. Current clinical data will be reviewed and with evaluation of the current recommendation of thyroid hormone suppression in the 2009 American Thyroid Association guidelines for thyroid nodules and cancer.

Nothing to Disclose: SLL

Pub # S48-1

Session Information SYMPOSIUM SESSION: TRANSLATIONAL - Yin & Yang of the Insulin-Like Growth Factor System as a Treatment Strategy (9:30 AM - 11:00 AM)

Title Rational for Targeting IGF-I Receptor in Cancer

Author String AV Lee
University of Pittsburgh, Pittsburgh, PA

Body The insulin-like growth factor (IGF) system, is critical for normal growth, and has both implicated in the both aging and cancer. Many of the IGF signaling components are oncogenes that can transform mouse fibroblasts (e.g. IGF-IR and IRS-1). Recent transgenic mouse studies showing that both IGF-IR and IRSs can cause mammary tumors confirms their oncogenic potential. However, IGFs likely act as factors permissive for tumorigenesis and/or promote cancer progression, enhancing the propagation of carcinogen-initiated cells. Supporting this, genetic deletion of the liver IGF-I gene in mice impairs carcinogen or oncogene induced tumorigenesis.

Laboratory evidence implicating IGFs in cancer development and progression is supported by numerous clinical observations. Circulating levels of IGFs are associated with risk of many cancers, and IGF-IR is overexpressed and hyperactive in many human tumors. The abundance of laboratory and clinical evidence implicating IGFs in the development and progression of cancer has led to numerous strategies to target these pathways. The IGF system is being targeted at the level of IGF ligands and receptors. Blocking antibodies and small molecule tyrosine kinase inhibitors have shown preclinical activity and several have entered clinical trials. However, early encouraging results have been tempered by lack of efficacy in large studies, and signs of toxicity. As with other targeted therapies, it is clear that targeting IGF-IR will not succeed with a 'one-size fits all' approach, and some form of patient selection will be needed. There is thus a major drive to develop biomarkers predicting response to IGF-IF inhibitors.

Session supported by: Pfizer, Inc.

Disclosures: AVL: Research Funding, Bristol-Myers Squibb, Pfizer, Inc.

Pub # S48-2
Session Information SYMPOSIUM SESSION: TRANSLATIONAL - Yin & Yang of the Insulin-Like Growth Factor System as a Treatment Strategy (9:30 AM - 11:00 AM)
Title Insulin-Like Growth Factor Targeting in Cancer: Trials & Tribulations
Author String P Haluska
Mayo Clinic, Rochester, MN

Body Insulin-like growth factor (IGF) signaling plays a key role in the growth, survival and invasion of human cancers (1). In addition, the dysregulated IGF signaling has been implicated as a major factor in resistance to clinical useful therapies in the treatment of cancer, including cytotoxic chemotherapy, hormonal therapy, biological/targeted therapy and radiation therapy (2). As such, targeting the IGF pathway has been a major focus of novel therapy development in oncology. To date, most therapies investigated have been monoclonal antibody (mAb) antagonists against the IGF Type 1 receptor (IGF-1R). Though variation exists among the class of agents, anti-IGF-1R mAb therapies block IGF-1 and IGF-2 activation of IGF-1R and IGF-1R/Insulin Receptor (InsR) hybrids and downregulate receptor expression. Due to the blockade of the major IGF signaling receptor, avoidance of interactions with the closely related InsR and precedence in the field for receptor-targeted mAbs demonstrating clinical benefit, anti-IGF-1R mAb therapies dominated the early clinical investigations with IGF targeted therapy. Early clinical trials have demonstrated anti-IGF-1R mAb therapy to be tolerable as single agent therapy and in combination with standard therapies (2). After some early evidence of benefits, IGF targeting with anti-IGF-1R mAb has failed to demonstrate clear clinical benefits in multiple tumor types. In addition, concerns about the metabolic consequences of continued IGF-1R engagement by mAb therapy, including hyperinsulinemia, hyperglycemia and increases in growth hormone have also dampened enthusiasm for this approach (3). Data has also accumulated that both IGF-1R and InsR are important cancer-related IGF signaling (4). In addition to strategies to mitigate the metabolic consequences of anti-IGF-1R mAb therapy, current trials are also focusing on alternative strategies, such as tyrosine kinase inhibition of IGF-1R/InsR, anti-IGF-1/IGF-2 ligand mAb and combinations with downstream signaling inhibitors. While data emerging from these newer strategies are encouraging, it is yet to be seen if these approaches will have less metabolic liabilities, more clinical activity and renew enthusiasm for targeting the IGF pathway in cancer.

Session supported by: Pfizer, Inc.

- (1) Pollak M. *Nat Rev Cancer*. 2008 Dec;8(12):915-28.
- (2) Weroha SJ, et al. *J Mammary Gland Biol Neoplasia*. 2008 Dec;13(4):471-83.
- (3) Haluska P, et al. *Clin Cancer Res*. 2007 Oct;13(19):5834-40.
- (4) Belfiore A, et al. *Endocr Rev*. 2009 Oct;30(6):586-623.

Sources of Research Support: Mayo Clinic Breast SPORE CA116201-03; Mayo Clinic Ovarian SPORE CA136393; NIH K12 CA090628-05.

Nothing to Disclose: PH

Pub # S48-3

Session Information SYMPOSIUM SESSION: TRANSLATIONAL - Yin & Yang of the Insulin-Like Growth Factor System as a Treatment Strategy (9:30 AM - 11:00 AM)

Title The Vitronectin: Insulin-Like Growth Factor Complex as Treatment for Chronic Wounds

Author String Z Upton
Queensland University of Technology, Kelvin Grove, Australia

Body Topical administration of growth factors has displayed some potential in wound healing, but variable efficacy high doses and costs have hampered their implementation. Moreover, this approach ignores the fact that wound repair is driven by interactions between multiple growth factors and extracellular matrix (ECM) proteins. We have previously reported that complexes of IGF bound to the ECM protein vitronectin (VN) significantly enhance cellular responses essential for wound healing in a deep dermal partial thickness porcine burn model (1). We now report results from the first human clinical study of VN:IGFBP:IGF for the treatment of chronic venous ulcers. This study (n = 30) examined twice weekly topical treatment of venous ulcers with VN:IGFBP:IGF for 3 weeks in combination with current best practice (compression therapy). The patients (average age = 71 years) had been receiving compression therapy for an average of 9.5 months prior to enrolment. The duration of the ulcer prior to enrolment varied significantly, with a mean of 11.1 months. At the start of the trial the mean ulcer size was 6.55 cm² and at day 24 the mean size was 3.75 cm². Five ulcers healed completely within 24 days with the average area of all 30 wounds being 43% of the original ulcer size. The reduction in wound area was statistically significant (p<0.005, two-sided Wilcoxon sign-rank test). Further, we assessed this complex in several [ldquo]difficult to heal[rdquo] case studies. The wound healing response shown in the case studies demonstrates that this complex may be applied to several ulcer etiologies, such as venous leg ulcers, diabetic foot ulcers and pressure ulcers. Importantly, these results were obtained with low doses (nanograms) of growth factors. We also report the development of chimeric proteins that are able to recapitulate within a single molecular species the biological activity of the multi-protein vitronectin:IGF complex thereby conferring numerous manufacturing and hence cost advantages. The chimeric VN:IGF-I protein is currently being trialled in patients with venous ulcers and the data to date will be presented. The finding that low doses of the vitronectin:IGF formulation elicit effective clinical responses, combined with the new chimeric protein approach, bodes well for the development of a cost-effective growth-factor:ECM protein-based wound therapy.

Session supported by: Pfizer, Inc.

(1) Upton Z et al. J Investig Dermatol 2008; 128:1535

Sources of Research Support: Queensland University of Technology, Queensland State Government, Tissue Therapies Limited.

Disclosures: ZU: Ad Hoc Consultant, Principal Investigator, Founder, Tissue Therapies Ltd.

Pub # S51-1

Session Information SYMPOSIUM SESSION: CLINICAL - Use of Growth Hormone (GH) & GH-Releasing Hormone in Abdominal Obesity: Progress & Pitfalls (9:30 AM - 11:00 AM)

Title Use of Growth Hormone-Releasing Hormone To Reduce Visceral Fat

Author String SK Grinspoon
Massachusetts General Hospital, Boston, MA

Body Growth hormone is secreted in a pulsatile fashion, related in part to a number of influences, including somatostatin, growth hormone releasing hormone (GHRH) and ghrelin. Pulsatile GH secretion is reduced in conditions of excess visceral fat, with preserved GH pulse frequency, but reduced pulse amplitude and AUC. Reduced GH is independently associated with increased cardiovascular risk indices in obesity, including cIMT, controlling for traditional cardiovascular risk factors. Therefore augmentation of GH may be of benefit in obesity and conditions marked by excess visceral fat. Use of daily exogenous GH, typically for growth hormone deficiency, does not result in a pulsatile GH pattern. In contrast, use of GHRH to augment endogenous GH in visceral obesity, may better mimic the physiological pattern of GH secretion. Short-term studies demonstrated a clear effect of a novel GHRH¹⁻⁴⁴ analogue, tesamorelin, to augment endogenous GH pulsatility, without effects on insulin sensitivity by euglycemic, hyperinsulinemic clamp in lean and obese subjects. Tesamorelin has also been investigated in longer-term studies in a model of acquired lipodystrophy among HIV-infected patients. Patients with lipodystrophy have increased visceral fat, but normal to reduced subcutaneous fat. In this population, two large 6-month Phase III trials have shown significant effects of once daily dosed tesamorelin to reduce visceral fat on average 15% vs. placebo, while sparing any reduction in subcutaneous fat. In addition, lipids, particularly triglycerides improved in pooled analyses. Tesamorelin improved adiponectin compared to placebo and also improved quality of life parameters as a function of the reduction in visceral fat. Fasting glucose, 2-hour glucose and insulin levels were not affected, but a slight increase in HgbA1c was seen in response to tesamorelin compared to placebo. The most common side effect was erythema at the injection site. Extension data from the Phase III trials demonstrated that treatment with tesamorelin for 12 months reduced visceral fat by 17.5% compared to baseline and no further effects on glucose were seen. Information learned from the use of GHRH in acquired lipodystrophy may serve as a model for the use of GHRH to reduce visceral fat in non HIV-infected patients, with relative GH deficiency and increased central fat accumulation, in whom augmentation of endogenous GH may have beneficial effects. Clinical trials are ongoing to answer this question.

Session supported by: Lilly USA, LLC & Pfizer, Inc.

Disclosures: SKG: Investigator, Theratechnologies; Consultant, Serono, Theratechnologies.

Pub # S51-2

Session Information SYMPOSIUM SESSION: CLINICAL - Use of Growth Hormone (GH) & GH-Releasing Hormone in Abdominal Obesity: Progress & Pitfalls (9:30 AM - 11:00 AM)

Title Does Growth Hormone (GH) Replacement Improve Metabolic Syndrome in GH-Deficient Adults?

Author String DR Clemmons
University of North Carolina School of Medicine, Chapel Hill, NC

Body A disproportionate number of patients who have severe GH deficiency have metabolic syndrome. Analysis of the response to GH is complicated by the fact that different studies used different criteria to define metabolic syndrome and the prevalence of some features are differentially increased in patients with specific etiologies of GHD. In spite of these limitations, several studies indicate that GH therapy improves several of these parameters. The variable that improves to the greatest extent is waist circumference. Some studies show improvement in both systolic and diastolic blood pressure and some have demonstrated an increase in HDL cholesterol, although this is not uniformly present. Definitive evidence of decreased levels of triglycerides has not been forthcoming. With regard to adipokine secretion, most studies have shown long term GH administration results in minimal changes in adiponectin, leptin and resistin. For glucose metabolism the results are clearly dependent on GH dose. A low dose e.g. 0.1 mg/day was noted to improve glucose utilization. However in studies wherein the entire range of GH doses was utilized, fasting glucose increased in approximately 23% of patients during the first six months of therapy and then returned toward normal in all but 11% of patients. Only 6% of patients developed evidence of diabetes mellitus during this treatment interval and this also tended to improve over time. Although GH administration clearly increases insulin resistance in these patients, most do not develop impaired fasting glucose or diabetes. In summary prevalence of the characteristic features of the metabolic syndrome is increased in adults with GH deficiency. Institution of GH therapy is likely to improve waist circumference and in many patients it will raise HDL. The effects on triglycerides and blood pressure are variable and not consistent across studies. Some of this lack of consistency may be due to patient selection and GH dosage. The effects on glucose metabolism are complex and involve increasing insulin resistance and decreasing visceral adiposity. The combined effect of these two changes results in maintenance of glucose homeostasis in most patients although a small subset may develop impaired fasting glucose or overt diabetes which will require modification of dosage. Whether these changes in these parameters of metabolic syndrome will result in a benefit in terms of altering cardiovascular mortality has not been definitively determined.

Session supported by: Lilly USA, LLC & Pfizer, Inc.

Nothing to Disclose: DRC

Pub # S51-3

Session Information SYMPOSIUM SESSION: CLINICAL - Use of Growth Hormone (GH) & GH-Releasing Hormone in Abdominal Obesity: Progress & Pitfalls (9:30 AM - 11:00 AM)

Title Investigative Use of Growth Hormone in Abdominal Obesity

Author String J Svensson
Sahlgrenska University Hospital, Göteborg, Sweden

Body Hypopituitary patients with severe growth hormone (GH) deficiency (GHD) have multiple metabolic aberrations including accumulation of visceral fat mass and impaired cardiovascular risk factors. Most of these features of GHD can be reversed by GH replacement therapy. In abdominal obesity, GH secretion is blunted. The low GH secretion might accelerate visceral fat accumulation and contribute to some of the secondary metabolic consequences of abdominal obesity. The similarities between severe GHD and the metabolic syndrome have resulted in the hypothesis that GH treatment might be useful also in abdominal obesity. The investigational use of GH treatment in abdominal obesity will be reviewed. So far, the efficacy of GH treatment in abdominally obese subjects has been less impressive than that previously observed during GH replacement therapy in hypopituitary patients with severe GHD.

Session supported by: Lilly USA, LLC & Pfizer, Inc.

Nothing to Disclose: JS

Pub # S43-1

Session Information SYMPOSIUM SESSION: BASIC - Regulation of Transcription by Nuclear Receptors & Coregulators (9:30 AM - 11:00 AM)

Title Control of Estrogen Receptor Activity via Receptor Dynamics

Author String ET Alarid
University of Wisconsin, Madison, WI

Body All signaling pathways involve mechanisms of both activation and deactivation. Steroid signaling imposes unique challenges particularly with respect to deactivation. In contrast to cell surface signaling where receptors initiate a cascade and then are rapidly removed from the membrane, steroid receptors are active participants in mediating steroid effects on gene expression in the nucleus. The question then becomes how steroid signaling coordinates both destruction of receptors and activation of transcription. Destruction of ER α protein and other steroid receptors is carried out by the 26S proteasome. Serine 118 of ER α , a residue that is phosphorylated within minutes of ligand binding, is essential for both maximal transcriptional activation and receptor degradation. However, phosphorylation is reported to both stabilize and degrade receptor while activating transcription. Our recent studies reveal new insight into a regulatory mechanism by which phosphorylation at S118 can lead to diverse functional outcomes. Using NMR and *in vitro* approaches, we provide evidence for a phosphorylation-induced structural change in the ER α N-terminal domain (NTD) which permits recruitment of the binding partner, the peptidyl prolyl isomerase Pin1. Pin1 induces further conformational changes in the NTD, resulting in a shift between two isomeric forms of pS118-ER α , *cis* and *trans*. Shifting the equilibrium between isomers by manipulation of Pin1 levels reveals roles for Pin1-directed changes in regulation of ER α protein stability and transcription mediated through the NTD and AF1. We will put forth a new model wherein ER α activity is mediated through distinct structural isoforms of phosphorylated ER α that allows receptors to be segregated between degradation and activation pathways.

Sources of Research Support: McArdle Laboratories for Cancer Research, NIH grants: T32GM08688 (PR), R01GM56230 (to K.P.L.), 1U54 GM074901(JLM), and P41 RR02301 and P41 GM66326 (National Magnetic Resonance Facility at Madison, JLM).

Nothing to Disclose: ETA

Pub # S43-2

Session Information SYMPOSIUM SESSION: BASIC - Regulation of Transcription by Nuclear Receptors & Coregulators (9:30 AM - 11:00 AM)

Title Coactivator CARM1-Mediated Signaling Crosstalk of the Estrogen Receptor α : Mechanism & Implications for Breast Cancer

Author String D Picard
University of Geneva, Geneva, Switzerland

Body The transcriptional activity of the estrogen receptor α (ER α) is induced by both estrogens and a large variety of other extracellular signals. We discovered the mechanism of activation of unliganded ER α by cAMP. The phosphorylation of the arginine methyltransferase CARM1 by protein kinase A (PKA) at a single serine is necessary and sufficient for direct binding to a novel regulatory groove of ER α , and the interaction is necessary for cAMP activation of ER α . Since the enzymatic activity of CARM1 is dispensable, we speculate that it might serve as a pioneer factor on ER α to promote the assembly of a signal-specific transactivation complex.

Sustained PKA activity contributes to tamoxifen resistance of breast cancer cells by allowing ER α to be activated by the partial antagonist tamoxifen. Surprisingly, in an apparent contradiction of the established role of the molecular chaperone Hsp90 for steroid receptor maturation, both the activation of ER α by cAMP and tamoxifen resistance depend on its recruitment. In addition, this requires the Hsp90 regulator HDAC6, whose expression is elevated by cAMP. Thus, this pathway to tamoxifen resistance, apart from providing exciting new mechanistic insights, opens new avenues for therapeutic interventions to restore tamoxifen sensitivity to some breast cancers.

Assuming that there might be yet other unknown ER α regulators, we have undertaken genome-wide screens both in yeast and in mammalian cells. Several potential ER α regulators are currently investigated, notably also for their potential involvement in tamoxifen-resistant breast cancer.

Nothing to Disclose: DP

Pub # S43-3

Session Information SYMPOSIUM SESSION: BASIC - Regulation of Transcription by Nuclear Receptors & Coregulators (9:30 AM - 11:00 AM)

Title Nuclear Corepressors & Thyroid Hormone Action

Author String AN Hollenberg
Beth Israel Deaconess Medical Center, Boston, MA

Body The nuclear corepressors, NCoR and SMRT, play a key role in thyroid hormone (T3) signaling based on their ability to interact with the unliganded thyroid hormone receptor (TR) isoforms in vitro. Thus, they have been presumed to play a vital role in hypothyroidism and, potentially, resistance to thyroid hormone (RTH) where the action of the unliganded TR is paramount. However, recent in vivo studies using corepressor-deficient mouse models demonstrate that NCoR and SMRT are also key regulators of T3-dependent TR signaling. Therefore the nuclear corepressors appear to play a vital role in T3-sensitivity both peripherally and centrally and also in disease states where the unliganded TR plays a role. A further understanding of the actions of nuclear corepressors in vivo will allow for new insight into T3-signaling in health and disease.

Nothing to Disclose: ANH

Pub # S44-1

Session Information SYMPOSIUM SESSION: TRANSLATIONAL - Basic Mechanisms of Mitochondrial Insulin Action (9:30 AM - 11:00 AM)

Title Increased Fatty Acid Oxidation Is Not Obligatory Linked to Reduced Adiposity

Author String GJ Cooney
Garvan Institute of Medical Research, Sydney, Australia

Body A prolonged period of positive energy balance results in obesity and reducing body fat relies on decreased energy intake and/or increased energy expenditure. Because decreased food intake (starvation) and prolonged exercise are associated with increased fatty acid oxidation, it is often presumed that increasing fat oxidation will automatically result in fat loss. There is however, little mechanistic evidence showing that a switch to fatty acid oxidation (in the absence of any change in energy expenditure or food intake) is sufficient to reduce adiposity. In animals, fatty acid oxidation can be acutely increased by pharmacological activation of AMPK to phosphorylate and inhibit acetyl-CoA carboxylase 2 (ACC2), or chronically increased by genetic deletion of ACC2. Both these manipulations reduce malonyl CoA levels and remove inhibition of carnitine palmitoyl transferase-1 (CPT-1) thereby accelerating fatty acid transfer to the mitochondria for oxidation. Our data shows that these strategies both increased whole body fatty acid oxidation without altering energy expenditure or adiposity (1). In isolated rat skeletal muscle, AMPK activation increased fat oxidation but reduced glucose oxidation indicating substrate switching rather than any change in energy expenditure. In the ACC2 knockout mice with a chronic increase in fat oxidation and no change in energy expenditure or food intake, glucose was diverted into glycogen storage in muscle and lipid synthesis in liver and adipose tissue. More recent studies in ACC2 knockout mice suggest that chronic pharmacological activation of AMPK may have effects to reduce adiposity by increasing mitochondrial capacity or influencing food intake but these actions appear to be independent of any effect of AMPK mediated by ACC2. Therefore increased energy expenditure (or decreased energy intake) appears to be essential for fat loss and increasing fatty acid oxidation alone has little effect to reduce adiposity.

(1) Hoehn KL, Turner N et al., Cell Metab 2010; 11:70

Sources of Research Support: NHMRC Program Grant #535921; Pfizer P3 Research Grant.

Nothing to Disclose: GJC

Pub # S44-2

Session Information SYMPOSIUM SESSION: TRANSLATIONAL - Basic Mechanisms of Mitochondrial Insulin Action (9:30 AM - 11:00 AM)

Title Epigenetic Regulation of Metabolic Diseases

Author String Y Ogawa
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Body Epidemiological and animal studies have suggested that metabolic diseases in adulthood may be acquired during fetal and neonatal events. We previously reported a mouse model in which offspring subjected to fetal undernutrition, when fed a high-fat diet, develop pronounced weight gain and impaired glucose metabolism as a result of a premature onset of neonatal leptin surge (1). On the other hand, it is reported that rearing rodents in small litters leads to overfeeding during the suckling period, thereby resulting in increased adiposity, hyperinsulinemia and altered lipid profiles in adulthood. The metabolic phenotypes, therefore, may be determined by epigenetic mechanisms, which are related to environmental factors, including nutritional status in early life, during pregnancy, and/or after birth.

DNA methylation is a key epigenetic contributor to the maintenance of gene silencing. We reported that expression of *Dnmt3a*, a *de novo* DNA methyltransferase gene, is increased in obese adipose tissue (2). On the other hand, feeding the mother methyl-supplemented diet during the suckling period has resulted in the improvement of high-fat diet-induced fatty liver and impaired glucose and lipid metabolism in adult offspring. These findings suggest the potential role of DNA methylation in adult metabolic diseases. It is noteworthy that neonates predominantly utilize lipids from milk as the energy source. After weaning, they utilize glucose as the energy source. During the suckling period, when fat intake is high, the rate of hepatic *de novo* lipogenesis is very low, but it increases with the onset of weaning and diminished intake of milk. We have recently found that expression of *glycerol-3-phosphate acyltransferase 1 (GPAT1)*, which encodes a rate-limiting enzyme for *de novo* lipogenesis in the liver, is markedly increased in response to the physiologic demand of lipogenesis during the weaning period. This may be related to the decreased DNA methylation of the *GPAT1* promoter. Interestingly, feeding dams with a high-calorie diet before and during pregnancy and suckling, which is known to increase fatty liver in pups, has caused decreased DNA methylation of the *GPAT1* promoter, increased *GPAT1* expression, and increased triglyceride levels in the liver of new born pups. Our data suggest that environmental factors such as nutritional status affect DNA methylation and metabolic diseases.

(1) Yura S et al., Cell Metabolism 2005; 1:371

(2) Kamei Y et al., Obesity 2010; 18:314

Sources of Research Support: The Japanese Ministry of Education, Culture, Sports, Science, and Technology; The Japanese Ministry of Health, Labour, and Welfare.

Nothing to Disclose: YO

Pub # S44-3

Session Information SYMPOSIUM SESSION: TRANSLATIONAL - Basic Mechanisms of Mitochondrial Insulin Action (9:30 AM - 11:00 AM)

Title Mitochondrial Dysfunction in Obesity & Diabetes: Chicken or Egg?

Author String ME Patti
Joslin Diabetes Center/Harvard Medical School, Boston, MA

Body The association of insulin resistance and risk for type 2 diabetes (DM) with obesity and inactivity indicates an important, and potentially pathogenic, link between fuel and energy homeostasis and the emergence of metabolic disease. Given the central role for mitochondria in fuel metabolism, it is possible that alterations in cellular mitochondrial oxidative function might contribute to the pathogenesis of type 2 DM. Consistent with this hypothesis, analysis of genomic, metabolomic, and in vivo data has demonstrated evidence for alterations in oxidative metabolism in individuals at risk for DM. Dysregulated metabolism in this setting may lead in turn to chronic accumulation of lipid oxidative metabolites and reactive oxygen species which can impair insulin signaling and increase insulin resistance. However, a causal pathogenic relationship remains uncertain. Our laboratory studies are focused on identification of gene expression and metabolomic patterns in individuals at high risk for diabetes, prior to the onset of disease. We will present data derived from these analyses to highlight potential pathways linked to induction of oxidative dysfunction and insulin resistance.

Nothing to Disclose: MEP

Pub # S45-1

Session Information SYMPOSIUM SESSION: TRANSLATIONAL - Genetics & Epigenetics of Pituitary Tumors (9:30 AM - 11:00 AM)

Title Epidemiology & Genetics of Familial Pituitary Adenomas

Author String AM Beckers
Centre Hospitalier Universitaire de Li[egrave]ge Sart Tilman, Liege, Belgium

Body Pituitary adenomas represent about 15% of primary intracranial neoplasms and have a prevalence of 1/1000 inhabitants in cross-sectional case-finding studies in developed countries such as Belgium and the United Kingdom (1,2). Most arise in a sporadic setting with >5% developing as a part of familial syndromes, predominantly MEN1 and as part of familial isolated pituitary adenomas (FIPA), while they also occur in other rarer hereditary conditions like Carney complex and MEN4 (3,4,5). Mutations in *MEN1* and *PRKARIA* genes are found in the majority of MEN1 and CNC patients respectively (5). About 15-20% of FIPA kindreds have mutations in the *AIP* gene (6,7). Mutations in the *CDKN1B* gene, encoding p27^{Kip1} were identified in MEN4 cases (8).

Familial pituitary tumors may have specific clinical characteristics. In MEN1 and FIPA they are more aggressive and affect patients at younger age, therefore justifying the importance of early recognition. *AIP* mutation-related pituitary adenomas have a particularly early onset (50% in children/adolescents) and there is a large predominance of somatotropinomas (80%; gigantism is frequent), followed by mixed GH/PRL tumors prolactinomas and non-secreting tumors. These tumors are usually very large at diagnosis and appear to be more frequently resistant to pharmacological treatment (9).

Based on the now-established clinical characteristics of *AIP* mutation related pituitary adenoma patients, it is reasonable to assess affected members of FIPA kindreds for *AIP* mutations. Patients with apparently sporadic aggressive adenomas, diagnosed at age <30 years, is a clinically delimited population that is enriched for *AIP* mutation positive cases, and in whom unrecognized familial mutation carriers are often noted. However, there is a need for a pragmatic clinical approach to this developing area of research. Such an approach would seek to avoid widespread genetic testing in unselected sporadic pituitary adenoma populations, as the likelihood of identifying *AIP* mutations is low. The risk of subsequent pituitary tumor development in unaffected *AIP* mutation carriers remains unclear, so unnecessarily invasive follow-up in the absence of good clinical evidence should be avoided. Regular clinical review and the opportunity for hormonal assessments should be considered and an initial MRI may be discussed with the patient. The optimal interval for follow up hormonal surveillance remains to be determined in longitudinal studies.

Daly AF et al. J Clin Endocrinol Metab 2006 91(12), 4769-4775
2. Fernandez A et al. Clin Endocrinol 2010;72(3):377-82.
Valdes Socin H et al. Ann Endocrinol-Paris. 2000;61:301.
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Pellegata NS. et al Proc. Natl. Acad. Sci. USA 2006 103(42), 15558-15563
Daly AF. et al J Clin Endocrinol Metab 2010 Nov;95(11):E373-83

Nothing to Disclose: AMB

Pub # S45-2

Session Information SYMPOSIUM SESSION: TRANSLATIONAL - Genetics & Epigenetics of Pituitary Tumors (9:30 AM - 11:00 AM)

Title The Role of AIP in Pituitary Tumorigenesis

Author String M Korbonits
Queen Mary University of London, London, UK

Body Germline mutations in the aryl hydrocarbon receptor interacting protein (AIP) gene have been described in 15-20% of patients from familial isolated pituitary adenoma families (FIPA), an autosomal dominant condition with incomplete (~30%) penetrance. AIP mutation families have a distinct clinical phenotype compared to AIP mutation negative FIPA families. They have young-onset, usually aggressive, mostly GH and/or PRL-secreting adenomas, with poor response to somatostatin analogue therapy, a male predominance (66%), and a slightly higher penetrance than AIP-negative families. In several families a clear pattern suggesting a founder effect has been observed (1). Germline AIP mutations have also been identified in apparently sporadic pituitary adenoma cases (GH, PRL and ACTH-secreting adenomas) cases, most often in young-onset cases. As a result of genetic screening followed by clinical assessment we have now prospectively identified several cases of macro- and microadenomas where early diagnosis and intervention was achieved.

AIP is molecular chaperone with classical tetratricopeptide domains important to binding to its numerous binding partners, including several nuclear receptors, heat-shock protein 90, phosphodiesterases and survivin. It is currently unknown as to what is the exact mechanism of its tumour suppressor role: 75% of mutations lead to a truncated protein, while missense mutations mostly affect important conserved residues in the TPR structure (2). Mutant AIP does not interact with protein binding partners and does not reduce cell proliferation in cell culture, as opposed to wild-type protein. In turn, down-regulation of endogenous AIP expression leads to increased cell proliferation. Micro-RNA regulation of AIP has also been suggested (3). Clinical data suggest down-regulation of AIP in sporadic aggressive somatotropinomas with aberrant expression profiles in gonadotroph and corticotroph adenomas. Interestingly, no somatic mutations have been described in pituitary adenomas, or indeed other studied tumour types. A number of questions remain open. Why do AIP mutations only cause pituitary adenomas? What is the mechanism of tumour suppression and the lack of responsiveness to somatostatin analogues? And what influences disease penetrance?

(1) Chahal et al., N Eng J Med 2011; 364:43
(2) Igreja et al., Human Mutation 2010; 31:950
(3) Trivellin et al., ENDO 2011

Nothing to Disclose: MK

Pub # S45-3

Session Information SYMPOSIUM SESSION: TRANSLATIONAL - Genetics & Epigenetics of Pituitary Tumors (9:30 AM - 11:00 AM)

Title Epigenetics of Pituitary Tumors

Author String WE Farrell
University Hospital of North Staffordshire/University of Keele, Stoke-on-Trent, UK

Body In common with multiple other tumor types pituitary adenomas harbour frequent and multiple epigenetic aberrations. These changes, imposed upon the genome post-replication, frequently lead to the silencing of genes with roles in development, cell cycle progression, apoptosis and receptors associated with these pathways. In addition to epigenetic changes, apparent as methylation of promoter associated CpG islands; histone tail modifications also play important roles in gene silencing. The role of these frequently interlinked changes, as determined by candidate gene and whole genome analyses, will be reviewed. More recently, investigations, in this and other tumor types, have exploited pharmacological unmasking to identify novel genes and explored their potential in clinical management strategies. In recent studies we explored the epigenetic profile associated with expression of the dopamine D2 receptor (D2R) in pituitary cells. Our studies showed, in cells that fail to express D2R an increase in promoter-associated CpG island methylation accompanied by enrichment for a histone mark associated with gene silencing, H3K27me3. Pharmacological unmasking strategies of GH3 cells, with a demethylating agent and a histone deacetylase inhibitor, led to decrease in CpG island methylation and concomitant enrichment for a histone mark associated with active genes, H3K9Ac. The unmasking led to re-expression of endogenous D2R in these cells and was associated with a significant, and specific increase in apoptosis indices to challenge with either Dopamine or its analogue Bromocriptine. These studies point to the potential efficacy of combined treatment with epigenetic drugs and dopamine agonists for the medical management of different pituitary tumour subtypes, resistant to conventional therapies.

Sources of Research Support: Research project award (WEF) from the Samantha Dickson Brain Tumour Trust (SDBTT).

Nothing to Disclose: WEF

Pub # S46-1

Session Information SYMPOSIUM SESSION: TRANSLATIONAL - Mechanotransduction in Bone (9:30 AM - 11:00 AM)

Title Mechanical Signals as Anabolic Agents in Bone

Author String JE Rubin
University of North Carolina, Chapel Hill, NC

Body Skeletal remodeling involves recruitment of new osteoblasts from multipotent mesenchymal stem cells (MSC) resident in the bone marrow. Mechanical signals of both low and high intensity are inhibitory to fat and anabolic to bone in vivo, and have been shown to directly affect the MSC pools from which fat and bone precursors emerge. To understand the signaling pathways utilized by mechanical input, we have studied bone marrow MSC cultured under conditions which promote adipogenesis. Daily treatments with either low (vibration) or high (high magnitude strain) mechanical inputs constrain adipogenesis, maintaining MSC potential for osteoblast recruitment. The signaling pathway by which mechanical input prevents adipogenesis involves inhibition of GSK3 β , which promotes increases in cytosolic and nuclear β -catenin. Interruption of β -catenin action blocks the ability of mechanical input to prevent adipogenesis. Mechanical inhibition of GSK3 β is Wnt independent, and requires proximal activation of AKT. In considering that mechanical inhibition of GSK3 β is transient, we found that if we reapplied the mechanical stimuli after a rest period of at least 1 h, the second application resulted in an enhanced signaling response measured by increased AKT activation and increased GSK3 β inhibition. Importantly, twice daily mechanical treatment allowed a reduction in mechanical input in both total cycles and magnitude, while further enhancing the end point, i.e., inhibition of MSC adipogenesis. Our continuing studies suggest that MSC adapt to mechanical input with alterations in cytoskeletal architecture, an adaptation which we believe primes the cell for a heightened response to physical stimuli. Our data lead us to suggest that incorporating several brief mechanical challenges within a day may be superior to a single large/long exercise treatment in terms of regulating MSC output. Understanding how mechanical input controls MSC differentiation should allow us to maximize exercise regimens which promote skeletal health.

Session supported by: Lilly USA, LLC

Sources of Research Support: NIH grants AR042360 and AR056655 to JR.

Nothing to Disclose: JER

Pub # S46-2

Session Information SYMPOSIUM SESSION: TRANSLATIONAL - Mechanotransduction in Bone (9:30 AM - 11:00 AM)

Title Dendritic Processes & Cell Body Responses of Osteocytes to Mechanical Loading

Author String JX Jiang
University of Texas Health Science Center, San Antonio, TX

Body Mechanical loading plays an essential role in bone formation and remodeling. Osteocytes with long dendritic processes embedded inside the bone tissue are mechanical sensory cells. Mechanical signals sensed by osteocytes are converted to chemical signals and convey these signals to other bone cells important for the process of bone remodeling. There has been a long-standing debate with regard to which part(s) of osteocyte, the cell body versus the dendritic process, acts as a mechanical sensor. To address this question experimentally, we used a transwell filter system that differentiates the cell body from the dendritic processes. Mechanical loading was applied to either the cell body or the dendrites, and the osteocyte's response was observed through connexin 43 (Cx43) hemichannel opening. The hemichannels located on the cell body were induced to open when mechanical loading was applied to either the dendritic processes or the cell body. However, no significant hemichannel activity in the dendrites was detected when either part of the cell was mechanically stimulated. The glycocalyx present in the extracellular matrix has been reported to act as a transducer of mechanical signals in other cells. We found that disruption of the glycocalyx by hyaluronidase on the dendrite side alone was sufficient to diminish dendrite's ability to induce the opening of hemichannels on the cell body, while hyaluronidase had no such effect when applied to the cell body. Importantly, hyaluronidase treatment to the dendrite side resulted in formation of poor integrin attachments with the reduced ability of the dendrites to form integrin attachments on the underside of the transwell filter. Together, our study suggests that the glycocalyx of the osteocyte dendritic process is required for forming strong integrin attachments. These integrin attachments probably serve as the mechanotransducers that transmit the mechanical signals to the cell body leading to the opening of connexin hemichannels, which permits rapid exchange of factors important for bone remodeling.

Session supported by: Lilly USA, LLC

Sources of Research Support: NIH grant AR46798 and Welch Foundation grant AQ-1507.

Nothing to Disclose: JXJ

Pub # S46-3

Session Information SYMPOSIUM SESSION: TRANSLATIONAL - Mechanotransduction in Bone (9:30 AM - 11:00 AM)

Title The Osteocyte: Orchestrator of Adaptive & Reparative Remodeling

Author String E Seeman
Austin Health, Melbourne, Australia

Body Genetic and environmental factors influencing the attainment of bone's peak material and structural strength during growth and its age-related decay do so through a final common pathway; bone modeling and remodeling. This cellular machinery is surface dependent - it adds and removes bone upon its outer (periosteal) and inner (endosteal) three (intracortical, endocortical, trabecular) surfaces fashioning and refashioning (adapting) bone's external size, shape and internal architecture in response to even herculean loads, and replacing matrix microdamage with an equal volume of new bone - successfully during growth and early adulthood.

This adaptive and reparative remodeling is, in large part, orchestrated by osteocytes interconnected by their many dendritic processes, a signal transduction system contained within a lacunocanalicular jungle so dense that each unit volume of bone is no more than a few microns from an osteocyte.

Osteocyte ablation produces bone fragility and failed mechano-transduction. Estrogen deficiency, corticosteroids and matrix microdamage produce osteocyte apoptosis, the demography of which identifies the location of microdamage which is relayed by the dendritic processes connected to endosteal surface lining cells. These cells become the roof of a focal bone remodeling compartment within which osteoclast and osteoblast precursors are recruited from marrow and elsewhere and differentiate into mature cells which excavate and replace damaged matrix with new bone. Osteocyte apoptosis precedes osteoclastogenesis. Damaged MLO-Y4 osteocyte-like cells secrete M-CSF and RANKL suggesting osteocytes participate in osteoclastogenesis. Sclerostin from osteocytes inhibits bone formation and its inhibition increases bone formation suggesting that osteocytes participate in the regulation of the volumes of bone resorbed and formed during remodeling.

Advancing age is associated with reduced periosteal bone formation, unbalanced and accelerated remodeling so adaptive and reparative modelling and remodeling become compromised. Reparative remodeling produces structural decay compromising bone strength more than the damage to be removed. Bone fragility is a disorder of the this cellular machinery and perhaps its orchestral leader; the osteocyte and its products are new therapeutic targets.

Session supported by: Lilly USA, LLC

Nothing to Disclose: ES

Pub # S49-1

Session Information SYMPOSIUM SESSION: CLINICAL - On the Quest for the Artificial Pancreas (9:30 AM - 11:00 AM)

Title Continuous Glucose Sensors: The Foundation for the Artificial Pancreas

Author String RA Vigersky
Walter Reed Army Medical Center, Washington, DC

Body The artificial pancreas (AP) has three distinct elements: a continuous glucose monitor (CGM), an insulin pump, and a linking algorithm. CGM is the most critical of the three since it provides the underlying data by which the other two function and is arguably the most fragile. There are three critical elements of CGM that must be operating near-perfectly for the AP to work: point and trend accuracy, provision of uninterrupted information, and resilience under a wide variety of physiologic and pathologic conditions. One of the potential Achilles heels of CGM is that it is usually calibrated from a single glucose value and any error is carried through until the next calibration. Thus, CGM's accuracy is highly dependent on the accuracy of the meter. Glucose meters are not all equal and even the best of them is relatively inaccurate being required to be within 20% of the reference above 75 mg/dl and within 15 mg/dl below 75 mg/dl. Meter inaccuracy may be due to any one or more of the following factors: strip manufacturing variability, human factors in technique, and storage issues among others. Both glucose meters and CGM's are susceptible to errors commonly seen in patients who are ill including ketoacidosis, hypotension, and oxygen therapy which is why CGM is not recommended for use in hospitalized patients at this time. While CGM has the advantage over point glucose monitoring by providing trend data, it is less accurate than glucose meters under a variety of important circumstances including in the hypoglycemic range (due to a high signal-noise ratio) and during rapid change in glucose. In addition, the inherent lag-time of sensors mostly related to the blood-interstitial barrier must be considered in any algorithm directing insulin infusion. Furthermore, since CGM sensors have periods of unexpected significant errors (which can be due to bio-fouling from encapsulation of the sensor, decreased vascularization, sensor compression while sleeping, or dislodgement of the sensor) any AP system must have a default failsafe pump infusion rate for use at such times. Still unknown influences on CGM accuracy include the use of common medications such as anti-inflammatory medications and vitamin preparations containing Vitamin C and common co-morbid conditions such as renal failure, dialysis, and cardiovascular disease.

Session supported by: Medtronic Diabetes

Sources of Research Support: DexCom, Inc.

Disclosures: RAV: Principal Investigator, DexCom.

Pub # S49-2
Session Information SYMPOSIUM SESSION: CLINICAL - On the Quest for the Artificial Pancreas (9:30 AM - 11:00 AM)
Title Closed-Loop Systems or Artificial Pancreas: Limitations of Current Technologies
Author String WV Tamborlane
Yale University School of Medicine, New Haven, CT
Body The introduction of external continuous glucose monitoring systems, improvements in insulin pumps and the availability of well established controller algorithms have made closed-loop insulin delivery systems a practical reality. In this talk, we will review the progress that has been made in development of the first generation of artificial pancreas systems and the results of short-term inpatient testing. The challenges that are presented by use of the subcutaneous route of insulin delivery and potential strategies that are being examined to enhance system performance will be discussed. Ultimate translation to outpatient use of these devices in the treatment of T1D will depend on improvements in the systems that will facilitate patient use and ensure the safety of patients by minimizing the risk of over delivery of insulin do to a system malfunction. A stepwise, incremental approach in applying this technology in the outpatient management of T1D is likely to be followed.

Session supported by: Medtronic Diabetes

Disclosures: WVT: Advisory Group Member, Medtronic Minimed, Novo Nordisk; Speaker, Novo Nordisk.

Pub #	S49-3
Session Information	SYMPOSIUM SESSION: CLINICAL - On the Quest for the Artificial Pancreas (9:30 AM - 11:00 AM)
Title	Artificial Pancreas: Emerging Technology?
Author String	SA Brown University of Virginia, Charlottesville, VA
Body	Session supported by: Medtronic Diabetes

Pub # S50-1

Session Information SYMPOSIUM SESSION: CLINICAL - Progestins & Cardiovascular Health in Women (9:30 AM - 11:00 AM)

Title Progesterone, the Renin-Angiotensin-Aldosterone System & Vascular Function in Women

Author String EW Seely
Brigham & Women's Hospital, Boston, MA

Body The risk for cardiovascular disease (CVD) is lower in women than in men before age 50 and then accelerates in women. Much research emphasis has focused on the role of estrogen in the protection of premenopausal women against CVD. However, randomized trials of estrogen treatment have not demonstrated a cardiovascular benefit. Progesterone, the other primary gonadal steroid produced by the ovary of premenopausal women, has received little attention as to its role in CVD and has important effects on the renin angiotensin aldosterone system (RAAS) and vascular function, both major contributors to CVD risk. Blockade of the mineralocorticoid receptor is associated with improved cardiovascular outcomes. Natural progesterone displaces aldosterone from the mineralocorticoid receptor leading to naturesis (1). However, synthetic progestins differ widely in their antialdosterone effects with medroxyprogesterone having limited antagonistic activity and drospirenone having activity similar to natural progesterone (2). Furthermore, natural progesterone has important vasodilatory effects, a characteristic which is not shared uniformly with synthetic progestins. In studies of human endothelial cells, progesterone has been shown to increase synthesis of nitric oxide (NO) where medroxyprogesterone was devoid of this activity (3). In studies of postmenopausal women, medroxyprogesterone prevents the NO mediated vasodilation associated with estrogen administration (4) whereas our group has demonstrated that natural progesterone does not (5). We have also shown that synthetic progestin therapy is also associated with lower renal blood flow, and the magnitude of this effect varies with the type of progestin (6). In studies of postmenopausal women, we have demonstrated that endogenous levels of progesterone are inversely associated with the magnitude of the vasoconstrictor response to infused angiotensin II in both the systemic and renal vasculature (7). Studies of drospirenone treatment have demonstrated that it lowers blood pressure in postmenopausal women (8). These data suggest that endogenous progesterone and synthetic progestins may be important modifiers of RAAS activity and vascular function and resultant CVD risk in women. Further consideration of the effects of specific progestins used in postmenopausal hormone therapy may lead to the identification of safer regimens.

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Disclosures: EWS: Researcher, Bayer, Inc.

Pub # S50-2

Session Information SYMPOSIUM SESSION: CLINICAL - Progestins & Cardiovascular Health in Women (9:30 AM - 11:00 AM)

Title Progestins & Cardiovascular Risk in Postmenopausal Women: Innocent Bystander or Guilty by Association?

Author String NF Santoro
University of Colorado, Denver, School of Medicine, Aurora, CO

Body An understanding of the role of progestins in cardiovascular disease (CVD) risk in women is somewhat hampered by the lack of a comprehensive model for CVD in women. Compared to men, inflammatory/autoimmune factors as well as prevalent psychosocial factors (depression, stress) play a more dominant role in disease risk, as does a premenopausal lack of estrogen (E). While exogenous E increases CVD risk or is neutral in its effect, endogenous E remains protective against CVD in the postmenopause. Because of its smooth muscle relaxant, anti-inflammatory and immunosuppressive effects, progesterone (P) might be expected to mitigate CVD risk. Micronized P has been well studied (in the PEPI trial) with respect to intermediate biochemical markers of CVD and has the least overall adverse impact on circulating lipoproteins and lipids compared to synthetic progestins. CRP appears to increase with estrogen and this effect is unmitigated by P or any progestin. Among the synthetic progestins, medroxyprogesterone acetate (MPA) has been the most well studied and has sometimes been shown to attenuate E-related beneficial changes in intermediate markers of CVD risk and in some cases attenuates E-induced flow mediated vasodilation in experimental animal models. Norethindrone, didrogesterone and drospirenone have been partially studied with intermediate effects in between P and MPA. The clinical significance of these effects are not known. Clinical trial data from the Women's Health Initiative demonstrate relatively large differences in CVD risk and risk factors in women taking E alone compared to those taking E + MPA; however, baseline differences between these 2 groups of women are large and biologically significant and outcomes cannot be attributed to treatment assignment alone. Head-to-head randomized, clinical trials are lacking to allow for direct comparisons between P and other progestins. Strategies to minimize the potential adverse impact of progestins in postmenopausal women taking menopausal hormone therapy (MHT) include the local administration of progestin to the uterus via a levonorgestrel intrauterine system; however, limited information on outcomes is available at this time. Conclusions: The cardiovascular role of the progestin type in the management of postmenopausal women taking MHT remains unclear due to incomplete medical evidence. Attribution of excess risk to MPA or to the progestin component of MHT regimens is not warranted at this time.

Nothing to Disclose: NFS

Pub # S50-3

Session Information SYMPOSIUM SESSION: CLINICAL - Progestins & Cardiovascular Health in Women (9:30 AM - 11:00 AM)

Title Combined Oral Contraceptives & Cardiovascular Health in Premenopausal Women

Author String R Sitruk-Ware
Population Council, New York, NY

Body Estrogen and progestins have been used by millions of women as effective combined oral contraceptives. Its safety has been documented by years of follow-up and serious adverse events that may be related to their use are rare in the young population exposed to these agents. The balance between the benefits and the risks of contraceptive steroids is generally positive in particular when comparing to the risks of pregnancy and especially in women with risk factors. Cardiovascular and venous risk have been associated with surrogate markers such as lipoprotein changes, and coagulation factors induced by the synthetic steroids used in contraception.

The synthetic estrogen Ethinyl-Estradiol (EE) exerts a stronger effect than natural estradiol (E2) on hepatic metabolism including estrogen-dependent markers such as liver proteins. This stronger hepatic impact of EE has been related to its 17 α -ethinyl group which prevents the inactivation of the molecule. Due to its strong activity, administering EE via a non-oral route does not prevent its impact on liver proteins. In order to circumvent the metabolic changes induced by EE, newer products using more natural compounds such as estradiol (E2) and estradiol valerate (E2V) have been introduced, as well as new progestins closer to progesterone.

The synthetic progestins used for contraception are structurally related either to testosterone (T) (estranes and gonanes) or to progesterone (pregnanes and 19-norpregnanes). Several new progestins have been designed to bind more specifically to the progesterone receptor and to minimize side-effects related to androgenic, estrogenic or glucocorticoid receptor interactions. Dienogest (DNG), and drospirenone (DRSP) and the 19-norpregnanes including Nestorone[®] (NES), norgestrel acetate (NOMAc) and trimegestone (TMG) have been combined with estrogen either EE or E2 or estradiol valerate (E2V). Risks and benefits of the newer progestins used in contraception depend upon the type of molecular structure, the type and dose of estrogen associated in a combination and the route of administration. The lower impact of estradiol-based combination on metabolic surrogate markers may result in an improved safety profile, but only clinical outcomes are relevant to assess the risk and large surveillance studies are warranted to confirm this hypothesis. So far, the contraindications and warnings for use of current hormonal combinations also apply to the estradiol-based contraceptives.

Sources of Research Support: Grants of USAID and the NICHD.

Disclosures: RS-W: Chairman, Bayer Schering Pharma; Advisory Group Member, Bayer Schering Pharma, Merck & Co.

Pub #

Session Information

CONFERENCE EVENT: Cooking for Pleasure and Health: Moving Patients from Good Intention to Healthy Lifestyles (11:00 AM)

Title

Cooking for Pleasure and Health: Moving Patients from Good Intention to Healthy Lifestyles

Author String

Body

Pub #

Session Information

CONFERENCE EVENT: Cooking for Pleasure and Health: Moving Patients from Good Intention to Healthy Lifestyles (1:00 PM)

Title

Cooking for Pleasure and Health: Moving Patients from Good Intention to Healthy Lifestyles

Author String

Body

Pub #

Session Information

CONFERENCE EVENT: Cooking for Pleasure and Health: Moving Patients from Good Intention to Healthy Lifestyles (3:00 PM)

Title

Cooking for Pleasure and Health: Moving Patients from Good Intention to Healthy Lifestyles

Author String

Body

Pub # OR20-1
Session Information ORAL SESSION: BASIC - Molecular, Physiological & Pathological Interactions of Steroid Receptors with Coregulators & Pioneer Factors (11:15 AM-12:45 PM)
Title High Throughput Analysis of Estrogen Receptor-alpha Identifies Novel Factors Influencing Gene Regulation
Author String MJ Bolt, FJ Ashcroft, A Malovannaya, J Qin, MA Mancini
Baylor College of Medicine, Houston, TX

Body Estrogen receptor- α (ER) is a type I nuclear receptor important in normal reproductive development and diseases such as breast cancer and osteoporosis. Ligand-activated ER can regulate gene expression through binding of estrogen response elements primarily located upstream of target genes. To visualize the mechanisms involved in ER mediated gene regulation, we have created and validated a microscopy-based approach to quantify coregulator recruitment to an ER-occupied promoter locus (1,2,3) as part of a platform that is amenable to high content analysis and screening (HCA/HCS). This system exploits an engineered dual stable cell line harboring a microscopically-visible, multicopy integration of the ER-regulated prolactin promoter array and GFP-ER in HeLa (PRL-HeLa). The goal of the current project is to identify the full spectrum of ER coregulators in order to create a more complete picture of ER-regulated transcription in a cellular context. Further, we have performed an antibody screen of >1000 transcriptionally relevant antibodies by HCA, and have identified members of the mediator complex and other basal transcription machinery that target the PRL array following treatment to estradiol (10-8M), tamoxifen (10-8M) or bisphenol A (10-6M). In parallel, we have also performed GFP-ER immunoprecipitation in estradiol-treated (10-8M) PRL-HeLa extracts followed by mass spectrometry (IP-MS) to identify ER interactors. IP-MS identified known and novel interactors including SRC-3 and sirtuin, respectively. These screening methods coupled with a siRNA screen to look for functional consequences of loss of coregulators or nuclear receptors have identified multiple novel factors impacting the PRL array or ER stability. We have identified helicases, ubiquitination enzymes, and nuclear receptors as possible regulators of ER-regulated transcription. Future studies centering on ER ligands and endocrine disruptors will utilize HCA to differentially classify effectors of ER function at a mechanistic level whereby ER or CoR functions are regulated through direct or indirect interactions and/or post-translational modifications.

- (1) Sharp et al., J Cell Sci 2006
- (2) Berno et al., PloS One 2008
- (3) Ashcraft et al., Gene 2011

Sources of Research Support: NIH 5R01DK055622; The Susan Komen Foundation KG091198; John S. Dunr Gulf Coast Consortium for Chemical Genomics; SCCPR U54 HD-007495; P30 DK-56338; P30 CA-125123; NLM Grant No. T15LM007093.

Nothing to Disclose: MJB, FJA, AM, JQ, MAM

Pub # OR20-2

Session Information ORAL SESSION: BASIC - Molecular, Physiological & Pathological Interactions of Steroid Receptors with Coregulators & Pioneer Factors (11:15 AM-12:45 PM)

Title Distinct Role of FoxA1 in Modulation of Androgen and Glucocorticoid Receptor Function and Binding to Chromatin

Author String B Sahu, M Laakso, K Ovaska, S Hautaniemi, OA Janne
University of Helsinki, Helsinki, Finland; University of Helsinki, Helsinki, Finland

Body Androgen receptor (AR) and glucocorticoid receptor (GR) are ligand-inducible transcription factors that belong to the superfamily of nuclear receptors. Transcriptional regulation by these receptors is mediated in collaboration with other DNA-binding transcription factors. Genome-wide analysis of AR-binding sites (ARBs) and GR-binding sites (GRBs) in prostate cancer (LNCaP-1F5) cells by ChIP-seq revealed high enrichment of FoxA1 cis-elements adjacent to the ARBs and GRBs. FoxA1 has been shown to act as a pioneer factor for ER binding to chromatin, and it facilitates the ability of estrogen to activate transcription (1, 2). To understand the relationship between AR, GR and FoxA1, we used ChIP-seq to map genome-wide binding sites for these proteins on LNCaP-1F5 cell chromatin in both parental cells and cells depleted from FoxA1. Binding of AR and GR to chromatin is remarkably fluid and highly dependent on the concentration of FoxA1 protein. Transient depletion of FoxA1 led to significant re-distribution of ARBs and GRBs on LNCaP-1F5 cell chromatin, revealing that FoxA1 plays a dual role in regulating AR and GR binding and function. First, FoxA1 is a pioneer factor and guides AR and GR to bind to their cognate chromosomal sites that, however, represents only a subset of ARBs and GRBs. Second, FoxA1 depletion resulted in the appearance of a large number of new ARBs and GRBs which was linked to androgen or glucocorticoid regulation of nearby genes that were not dependent on these steroids in parental cells. Despite their almost identical cis-elements, only about one-fourth of new ARBs overlapped with new GRBs in FoxA1-depleted cells, indicating that local chromatin environment is important for binding site recognition by the receptor. This pattern of receptor binding was commensurate with androgen- or glucocorticoid-dependent transcription programs in parental and FoxA1-depleted cells. Our results are in sharp contrast to the role of FoxA1 in estrogen signaling in breast cancer cells where FoxA1 depletion abolishes both ER binding and estrogen-dependent gene expression (3). In the case of AR and GR signaling, FoxA1 is not only a pioneer factor, but it can also prevent binding of AR and GR to chromatin sites. Collectively, our results indicate that the steroid and the receptor are mandatory but not sufficient in most instances to guide the receptors to appropriate chromosomal locations, in order to regulate the intended transcription programs.

- (1) Carroll et al., Cell 2005; 122:33
(2) Lupien et al., Cell 2008; 132:958
(3) Hurtado et al., Nat Genet. 2011; 43:27

Sources of Research Support: CRESCENDO; Academy of Finland; Sigrid Juselius Foundation.

Nothing to Disclose: BS, ML, KO, SH, OAJ

Pub # OR20-3

Session Information ORAL SESSION: BASIC - Molecular, Physiological & Pathological Interactions of Steroid Receptors with Coregulators & Pioneer Factors (11:15 AM-12:45 PM)

Title Targeted Disruption of the p160 Coactivator Interface of Androgen Receptor (AR) Selectively Inhibits AR Activity in Both Androgen-Dependent and Castration-Resistant AR-Expressing Prostate Cancer Cells

Author String M Nakka, I Agoulnik, N Weigel
BCM, Houston, TX

Body Prostate cancer (PCa) is an androgen dependent disease; thus, androgen ablation is the primary treatment for metastatic PCa. Although tumors respond, most become resistant to treatment within two years. There is good evidence that castration resistant PCa also is dependent on the androgen receptor (AR). A variety of mechanisms have been proposed for the reduced requirement for androgens. Among these is the expression of constitutively active AR splice variants lacking the carboxyl terminal hormone binding domain. These findings highlight the need for alternate treatments targeting AR. We took advantage of the observation that the p160 coactivators interact most strongly with the amino-terminal AF-1 in AR. To examine the consequences of disrupting the interaction between p160 coactivators and AR, we expressed two peptides that are derived from the region of the p160 coactivator, SRC-1, that interacts with AR. Both interacted with AR as expected. When expressed using a mammalian expression vector, these peptides reduce AR dependent induction of an AR responsive reporter. Importantly, the peptides do not inhibit thyroid receptor, glucocorticoid receptor or estrogen receptor activity indicating that the peptides are selective. Further, these peptides interrupt the AR/SRC-1; AR/SRC-2 and AR N\C interactions, but not SRC-1/CARM-1 interactions measured using a mammalian two hybrid assay. To study the effect of these peptides on endogenous AR dependent target gene expression, peptide expressing vectors were transiently transfected into LNCaP cells. Consistent with the SRC-1 dependence of induced, but not repressed genes, the peptide inhibited hormone dependent induction of target genes including PSA and TMPRSS2, but did not block AR dependent repression of PCDH11Y and UGT2B17. Simultaneous detection of SRC-1 peptides and PSA by double immunofluorescence in transfected LNCaP cells clearly demonstrated a strong reduction in PSA levels in the cells expressing the peptides. Importantly, the peptides also inhibited the AR dependent expression of PSA in castration resistant C4-2 cells grown in androgen depleted medium. The peptides inhibited androgen dependent proliferation of LNCaP cells and proliferation of C4-2 cells in androgen depleted medium, but had no effect in AR lacking PC-3 cells. Thus, the p160 coactivator binding site is a potential therapeutic target to inhibit AR activity regardless of the mechanism of activation.

Nothing to Disclose: MN, IA, NW

Pub # OR20-4
Session Information ORAL SESSION: BASIC - Molecular, Physiological & Pathological Interactions of Steroid Receptors with Coregulators & Pioneer Factors (11:15 AM-12:45 PM)
Title Leveraging PARP1 Function as a Means to Control AR Signaling and Improve Prostate Cancer Management
Author String MJ Schiewer, J Goodwin, MA Augello, F Liu, JL Planck, JR Brody, JM Pascal, KE Knudsen
Thomas Jefferson University, Philadelphia, PA; Thomas Jefferson University, Philadelphia, PA; Thomas Jefferson University, Philadelphia, PA; Thomas Jefferson University, Philadelphia, PA; Thomas Jefferson University, Philadelphia, PA; Thomas Jefferson University, Philadelphia, PA

Body Poly (ADP-ribose) polymerase 1 (PARP1) is an abundant nuclear enzyme that modifies substrates by poly (ADP-ribose)-ylation. While PARP1 has a well-described role in repair of DNA damage (especially as associated with base excision repair), substantive evidence suggests that PARP1 has additional functions in transcriptional control. The transcriptional regulatory functions of PARP1 are multi-fold, do not universally require enzymatic activity, and are manifest through divergent functions including: enhancer binding, association with insulators, modulation of chromatin structure, and/or direct transcription factor regulation. Recent pre-clinical studies and pre-clinical trials have illuminated the importance of PARP1 in a human cancers, in that enzymatic inhibitors of PARP1 create synthetic lethal events in tumors deficient in PTEN or BRCA1/2, and/or synergize with DNA damaging agents. Given the poor response of prostatic adenocarcinomas (PCa) to DNA damaging agents, initial studies were performed to assess the impact of PARP1 inhibitors on the radiation and chemotherapy responses. Consistent with studies in breast cancer, PARP1 inhibition sensitizes cell model systems of multiple stages of PCa to DNA damaging agents. However, an unexpected finding was noted in that PARP1 inhibition exhibited a single agent effect in cells positive for the androgen receptor (AR). These findings were consistent in both cells responsive to androgen deprivation therapy (ADT), which is the first line of intervention for disseminated disease, as well as therapy-resistant cells. Mechanistic exploration revealed that PARP1 is a critical effector of AR activity, and is recruited to sites of AR function upon receptor activation. Suppression of PARP1 activity significantly impaired transcriptional activation of endogenous AR target genes, including PSA, which is used clinically to monitor prostate cancer growth and progression. Genetic deletion of PARP1 similarly compromised AR activity, and restoration of PARP1 functional domain mutants into this model system allowed for confirmation of the posit that PARP1 catalytic activity is essential for supporting AR activity. Collectively, these studies identify PARP1 as a key mediator of AR activity that could be targeted in advanced disease to both suppress AR signaling and improve response to therapeutic DNA damaging agents.

Sources of Research Support: NIH grants (CA099996 and CA116777 to K.E.K.), and DOD Pre-doctoral Fellowship (PC094195 to M.J.S.).

Nothing to Disclose: MJS, JG, MAA, FL, JLP, JRB, JMP, KEK

Pub # OR20-5

Session Information ORAL SESSION: BASIC - Molecular, Physiological & Pathological Interactions of Steroid Receptors with Coregulators & Pioneer Factors (11:15 AM-12:45 PM)

Title Cell Specificity of the PR Transcriptome Is Critically Influenced by Chromatin Structure and Key Transcriptional Cofactors

Author String CL Clarke, JD Graham
University of Sydney at Westmead Millennium Institute, Westmead, Australia

Body Progesterone is critical for normal reproductive tissue function and plays strikingly distinct roles in the tissues in which it acts. Its effects are mediated by its nuclear receptor, PR, which in response to hormone binds specific elements on DNA to regulate transcription. Where studied, the transcriptional responses to progesterone in differing normal and malignant cell types are quite distinct. We previously demonstrated that the progesterone-responsive transcriptomes in breast cancer cells and primary normal breast cell cultures are largely non-overlapping. The mechanisms underlying this divergent progesterone response are not well understood, but chromatin organization, PR expression and the complement and abundance of specific transcriptional cofactors are all likely to influence progesterone response. To determine whether differing patterns of PR chromatin interaction underlie the distinct profiles observed, we used genome-wide PR ChIP-seq to map PR interactions on genomic DNA in two cell lines: T-47D breast cancer cells and AB32, a PR positive clone of the MCF-10A minimally transformed normal breast cell line. Expression profiling of progestin-responsive genes was also performed in both cell lines. Significant PR binding was detected at over 6000 genomic sites in T-47D and 8000 genomic sites in AB32 cells. In both cell lines transcriptional regulation was correlated with genomic interaction, and PR binding regions were identified near a majority of progestin-regulated genes in both cell lines. Most PR binding near progestin-regulated genes occurred within 50kb of the transcription start in both cell lines (77% in T-47D, 61% in AB32). The overlap between genomic binding of PR in T-47D and AB32 cells was limited, and this was reflected in a limited overlap in transcriptional profiles in response to progestin. Exposure of AB32 to chromatin modifiers altered the profile of progestin response, showing that nuclear architecture shapes progestin response. PR binding regions in T-47D and AB32 revealed distinct patterns of motif enrichment, demonstrating that different cofactors were implicated in mediating the progestin response in the two cell lines. Our data show that chromatin structure and cofactor expression are critical determinants of PR fidelity in normal and breast cancer cells.

Nothing to Disclose: CLC, JDG

Pub # OR20-6

Session Information ORAL SESSION: BASIC - Molecular, Physiological & Pathological Interactions of Steroid Receptors with Coregulators & Pioneer Factors (11:15 AM-12:45 PM)

Title The Role of Smooth Muscle Cell Mineralocorticoid Receptors in Blood Pressure Regulation and Vascular Injury

Author String AT McCurley, NN Mohammad, H Nickerson, M Aronovitz, A Krust, D Metzger, P Chambon, ME Mendelsohn, IZ Jaffe
Tufts Medical Center, Boston, MA; Coll[grave]ge de France, Illkirch, France

Body Hypertension and vascular injury play critical roles in the pathophysiology of heart attack and stroke, two of the leading causes of mortality in developed countries. The steroid hormone aldosterone (aldo) regulates blood pressure (BP) by binding to renal mineralocorticoid receptors (MR). In clinical trials MR antagonists decrease cardiovascular mortality and prevent heart attacks and strokes by incompletely understood mechanisms. Our lab has demonstrated the presence of functional MR in human vascular smooth muscle cells (VSMC) and endothelial cells, supporting a potential role for direct vascular effects of aldo in regulating BP and vascular disease. In models of vascular injury, aldo treatment increases and MR antagonists inhibit vascular thickening and VSMC proliferation, supporting a role for MR in vascular injury. We have developed a novel mouse model that allows for inducible, SMC-specific, deletion of MR that can be used to test the role of SMC-MR in vascular physiology *in vivo*. *We hypothesize that MR activation in VSMC regulates vascular tone thereby contributing to systemic BP and promotes the vascular proliferative and fibrotic response to injury.* We first confirmed SMC-specific deletion of MR in this animal model. Tail cuff and telemetric BP measurements were then performed on SMC-MR deficient mice compared to MR-intact controls. These studies show no difference in BP in young (3-4 month-old) mice but aged SMC-MR deficient mice (9-10 months-old) have significantly lower BP compared to MR-intact controls. Aortic vessel contraction and relaxation studies are consistent with the age-dependent BP phenotype seen in tail-cuff and telemetry studies. To address the role of SMC-MR in aldo-stimulated vascular injury we performed carotid artery wire injury on SMC-MR deficient mice and MR-intact controls in the presence and absence of aldo. Deficiency in SMC-MR significantly reduced aldo-mediated SMC proliferation and extracellular matrix deposition following injury supporting a critical role for SMC-MR in the pathophysiology of vascular remodeling. These findings support a new role for SMC-MR in regulating BP and aldo-stimulated vascular injury and suggests novel mechanisms for the protective effects of MR antagonist drugs in clinical trials. This new paradigm provides a foundation for future work exploring drugs that inhibit SMC-MR as novel targets to prevent or treat hypertension, atherosclerosis and other common vascular diseases in humans.

Nothing to Disclose: ATM, NNM, HN, MA, AK, DM, PC, MEM, IZJ

Pub # OR21-1

Session Information ORAL SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Control of Reproductive Function & Dysfunction (11:15 AM-12:45 PM)

Title Genes Regulated by Ovarian Rigidity Are Associated with PCOS

Author String P Mutharasan, E Galdones, B Penalver Bernabe, OA Garcia, RS Legro, A Dunaif, LD Shea, TK Woodruff, M Urbanek
Northwestern University Feinberg School of Medicine, Chicago, IL; Northwestern University Feinberg School of Medicine, Chicago, IL; Northwestern University, Evanston, IL; Pennsylvania State University MS Hershey Medical Center, Hershey, PA

Body
Background: Polycystic ovary syndrome (PCOS), a common endocrine disorder in women, is a complex disease with a poorly understood pathogenesis. Recently, it has been proposed that increased rigidity in the ovarian environment perturbs follicular development, thereby contributing to PCOS. In support of this hypothesis, we have previously identified the gene for fibrillin-3, an extracellular matrix protein expressed in ovaries, as a PCOS susceptibility locus (1-3). To address this hypothesis in vitro, we cultured mouse follicles within permissive (soft) or non-permissive (rigid) three-dimensional hydrogel environments and identified a panel of differentially expressed genes via microarray analysis (4). To determine whether these experimentally identified candidate genes contribute to PCOS, we measured the association and genetic variation in a human cohort.
Methods: A case-control candidate gene analysis was performed in 976 Caucasian cases diagnosed by NIH criteria and 977 Caucasian controls. Using the Illumina iSelect platform, we tested multiple haplotype-tagged SNPs (n= 2283 SNPs within and near each of the 133 identified candidate genes, with minor allele frequency > 0.05 and r² > 0.8) along with known coding SNPs within the genes.
Results: The greatest associations were found with SNPs within the following genes: RAMP1 (receptor activity modifying protein 1; p = 0.0003), COL15A1 (collagen, type XV, alpha 1; p = 0.0004), ADAMTS19 (ADAM metalloproteinase with thrombospondin type 1 motif, 19; p = 0.0008), ATF3 (activating transcription factor 3; p = 0.0013), OOEP (oocyte expressed protein homolog; p = 0.0018), and LRP5 (low density lipoprotein receptor-related protein 5; p = 0.0018).
Conclusions: These results suggest that ovarian follicles are regulated not only by hormones but by their physical environment. Genes that respond to changes in physical stress represent a novel functional candidate group that may contribute to the pathogenesis and/or underlying polygenic character of PCOS thus meriting further study. COL15A1, which is found in the extracellular matrix of various tissues, may play a role in the etiology of PCOS by altering ovarian rigidity.

1. Urbanek M et al., J Clin Endocrinol Metab 2007; 92:4191
2. Urbanek M, et al., J Clin Endocrinol Metab 2005; 90:6623
3. Urbanek M et al., Proc Natl Acad Sci U S A 1999; 96:8573
4. Woodruff TK, Shea LD et al., J Assist Reprod Genet 2010; in press

Sources of Research Support: NIH grant 54HD041857 from the National Institute Of Child Health And Human Development awarded to EG, BPB, LDS, TKW, AD and MU; NIH grant R01HD057450 awarded to OAG and MU; NIH grant P50HD044405 from the National Institute Of Child Health And Human Development awarded to AD and MU; American Diabetes Association Career Development Award 7-09-CD-13 awarded to MU.

Nothing to Disclose: PM, EG, BPB, OAG, RSL, AD, LDS, TKW, MU

Pub # OR21-2

Session Information ORAL SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Control of Reproductive Function & Dysfunction (11:15 AM-12:45 PM)

Title Androgen Administration in Normal Reproductive-Age Women Is a Promoter of Diet-Induced Oxidative Stress as Characterized in Polycystic Ovary Syndrome (PCOS)

Author String F Gonzalez, KS Nair, E Basil, JK Daniels, HE Blair
Indiana University School of Medicine, Indianapolis, IN; College of Medicine, Rochester, MN

Body

Objective: In PCOS, there is increased protein expression of p47^{phox}, the key component of NADPH oxidase in the fasting state and in response to a dietary carbohydrate trigger. This pro-oxidant phenomenon directly correlates with circulating androgens. We examined the effect of oral androgen administration on p47^{phox} expression in mononuclear cells (MNC), and on plasma thiobarbituric acid-reactive substances (TBARS), a commonly used index of lipid peroxidation, in normal reproductive-age women. This effect was evaluated in the fasting state, and in response to glucose ingestion.

Design: Prospective, controlled study

Methods: Sixteen lean ovulatory women between ages 18-40 without evidence of androgen excess were selected for study. All subjects underwent a 2-hour glucose tolerance test before and after orally ingesting 130 mg of DHEA (n=8) or placebo (n=8) for 5 days, in a randomized double-blind fashion. p47^{phox} RNA and protein expressions were respectively determined by real-time quantitative PCR and Western blotting in MNC isolated from blood samples drawn fasting and 2 hours after glucose ingestion. TBARS was measured by fluorescence from the same blood samples.

Results: Before treatment, subjects receiving DHEA or placebo exhibited no significant differences in androgens, and the percent change (%[Delta]) in p47^{phox} RNA and protein expressions, and plasma TBARS before and after glucose ingestion. Compared to the placebo group, the DHEA group exhibited higher post-treatment levels of testosterone (123±9 vs. 45±4 ng/dl, p<0.0001) and androstenedione (2.2±0.1 vs. 1.5±0.1 ng/ml, p<0.002). After DHEA treatment, the %[Delta] p47^{phox} RNA expression increased compared to placebo in response to glucose ingestion (50.8±19.7 vs. -5.2±11.6, p<0.03) while the %[Delta] p47^{phox} protein expression increased compared to placebo in the fasting state (29.3±12.1 vs. -5.3±5.7, p<0.03), and in response to glucose ingestion (11.0±5.4 vs. -11.6±6.8, p<0.03). The %[Delta] TBARS was also increased after DHEA treatment compared to placebo in the fasting state (-11.0±9.2 vs. 12.9±4.2, p<0.04), and in response to glucose ingestion (18.7±6.9 vs. -6.3±8.2, p<0.04).

Conclusion: These preliminary data demonstrate increased p47^{phox} gene expression and increased circulating TBARS in normal reproductive-age women after raising circulating androgens to levels observed in PCOS. Thus, hyperandrogenemia is capable of activating MNC, and increasing MNC sensitivity to glucose in this population.

Sources of Research Support: NIH grant HD048535 to F.G.

Nothing to Disclose: FG, KSN, EB, JKD, HEB

Pub # OR21-3
Session Information ORAL SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Control of Reproductive Function & Dysfunction (11:15 AM-12:45 PM)

Title A Single Injection of Kisspeptin-54 Increases LH Pulse Amplitude and Pulse Frequency in Healthy Women

Author String CN Jayasena, AN Comminos, S Misra, A Abbara, M Donaldson, MA Ghatei, SR Bloom, WS Dhillon
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Body **BACKGROUND:** Kisspeptin is a novel hypothalamic hormone with powerful stimulatory effects on the hypothalamo-pituitary-gonadal (HPG) axis. Inactivating mutations in the kisspeptin receptor lead to pubertal failure (1). We have previously demonstrated that injection of kisspeptin-54 stimulates luteinising hormone (LH) release in healthy men and women (2,3). Recent studies in animals suggest that endogenous kisspeptin signalling may be involved in stimulating the GnRH pulse generator (4). Determining whether exogenous administration of kisspeptin can stimulate the human GnRH pulse generator, has important therapeutic implications.

AIM: To determine the effects of kisspeptin-54 administration on LH pulse amplitude and frequency in healthy female volunteers.

METHODS: Six healthy female volunteers underwent frequent blood sampling for serum LH measurement every 10min for 8 hours (as a surrogate marker of the GnRH pulse generator) during the follicular phase of menstrual cycle. A sc injection of saline or kisspeptin-54 (0.3-0.6nmol/kg) was administered 4 hours after commencing the study. LH pulsatility within each subject was compared between the 4 hours pre-injection and 4 hours post-injection using a modified Santen and Bardin analysis (5). All studies were ethically approved.

RESULTS: No significant differences in number of LH pulses and LH pulse amplitude were observed before and after saline injection. A 4-fold increase in mean number of LH pulses was observed following kisspeptin-54 injection when compared with the pre-injection period (mean number of LH pulses during 4 hours: 0.75 ± 0.48 , pre-injection; 3.00 ± 0.69 , post-injection, $P < 0.05$ vs. pre-injection). The mean LH pulse amplitude was nearly 2-fold higher after kisspeptin-54 injection when compared with the pre-injection period (mean pulse amplitude in iU/L during 4 hours: 1.31 ± 0.09 , pre-injection; 2.31 ± 0.25 , post-injection, $P < 0.05$ vs. pre-injection).

CONCLUSIONS: We demonstrate for the first time that a single injection of kisspeptin-54 modulates the pulsatile pattern of LH secretion in healthy women, by increasing LH pulse frequency and LH pulse amplitude. Our data therefore provide the first evidence suggesting that kisspeptin-54 stimulates activity of the GnRH pulse generator in humans. This data has important therapeutic implications for the future development of kisspeptin to treat patients with disorders of reproduction.

- (1) Seminara SB et al., *N Engl J Med* 2003; 349:1614-1627
- (2) Dhillon WS et al., *J Clin Endocrinol Metab* 2005; 90:6609-615
- (3) Dhillon WS et al., *J Clin Endocrinol Metab* 2007; 92:3958-3966.
- (4) Plant TM et al., *Endocrinology* 2006. 147:1007-13.
- (5) Adams JM et al., *J Clin Endocrinol Metab* 1994. 79:858-64.

Sources of Research Support: NIHR Clinical Lectureship (CNJ); Society for Endocrinology Early Career Grant (CNJ). HEFCE Clinical Senior Lecturer Award (WSD). MRC Experimental Medicine project grant; Integrative Mammalian Biology Capacity Building Award; NIHR Biomedical Research Centre Funding Scheme.

Nothing to Disclose: CNJ, ANC, SM, AA, MD, MAG, SRB, WSD

Pub # OR21-4

Session Information ORAL SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Control of Reproductive Function & Dysfunction (11:15 AM-12:45 PM)

Title Primary Ovarian Insufficiency Is Associated with a High Prevalence of New NOBOX Loss-of-Function Mutations

Author String J Bouilly, A Bachelot, I Broutin, F Jaubert, P Touraine, N Binart
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Body Primary ovarian insufficiency (POI) is a disorder associated with female infertility, which affects approximately 1% of women under 40 years of age. POI encompasses a heterogeneous spectrum of conditions, through two major mechanisms, follicle dysfunction and follicle depletion. Although causes such as autoimmunity, monosomy X, and environmental factors play a role in POI, the etiology remains unknown in most cases. A genetic component has been suggested as one possible cause of the majority of cases of nonsyndromic forms. NOBOX (Newborn Ovary Homeobox) is an ovary-specific gene, playing a critical role in the transition of primordial to primary follicles in mice, which absence leads to sterility mimicking a POI. Because NOBOX represents a good candidate gene for this pathology, we sequenced NOBOX in a cohort of 178 women with idiopathic primary ovarian insufficiency. Among 19 identified variations, we described 4 missense (G91W, R117W, S342T and V350L) and one R303X nonsense NOBOX heterozygous mutations in 12 patients which present heterogenous clinical phenotype, from absence of puberty to secondary amenorrhea. When wild-type and mutant proteins were coexpressed in HEK293T or COS-7 cells mimicking the situation of the heterozygous patients, we demonstrated that these mutations lead to impaired NOBOX transactivation activity of GDF9 promoter-reporter gene. This argues against a dominant negative effect of all these mutations in vivo. Moreover, we described for the first time NOBOX in human embryonic and adult oocytes as well as in granulosa cells. Our data suggest that mutated NOBOX is associated with a progressive loss of ovarian reproductive capacity in women. We show functional loss of NOBOX activity in POI women and the protein localization in women ovaries. This finding provides a human model for the loss of function of NOBOX, and the 6.2% prevalence found in our cohort constitutes evidence for the inclusion of NOBOX in the list of proteins involved in human POI and potentially leading to counselling genetics. This validates the pivotal role of the human NOBOX transcription factor in first steps of folliculogenesis and in the maintenance of the follicular pool.

Nothing to Disclose: JB, AB, IB, FJ, PT, NB

Pub # OR21-5

Session Information ORAL SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Control of Reproductive Function & Dysfunction (11:15 AM-12:45 PM)

Title TRKB Receptors Are Required for the Activation of a PI3K/AKT-Mediated Signaling Pathway Essential for Oocyte Survival

Author String MD Dorfman, C Garcia-Rudaz, B Xu, GA Dissen, SR Ojeda
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Body Tropomyosin-related kinase receptor B (TrkB), the high-affinity receptor for neurotrophin-4/5 and brain-derived neurotrophic factor (BDNF), is required for follicular assembly and early follicular growth. Alternative splicing generates both a full length receptor (TrkB-FL) and truncated isoforms (TrkB-T1 and TrkB-T2), lacking the intracellular domain. Using a Cre-loxP recombination strategy to delete the *TrkB* gene exclusively in oocytes, we observed that these mutant animals (referred to as *OoTrkB*^{-/-} mice) become infertile in early adulthood, and that this deficit is associated with post-pubertal oocyte death and loss of follicular organization. Oocytes of immature ovaries express only TrkB-T receptors, and yet they are perfectly healthy in *OoTrkB*^{-/-} mice until after the first ovulation, suggesting that their death may be related to events initiated by the first preovulatory surge of gonadotropins. Mimicking this surge by treating wild-type mice with PMSG followed 48h later by hCG, did not alter TrkB-T abundance, but resulted in the appearance of immunoreactive TrkB-FL protein in oocytes as early as 4 h after hCG, with levels decreasing 24h post-injection in oocytes that remained in the ovary after ovulation. In contrast, TrkB-FL remained undetectable in *OoTrkB*^{-/-} mice after gonadotropin stimulation. In neural cells, TrkB-FL receptors support cell survival by activating a PI3K/AKT-mediated pathway, and ligand-dependent activation of TrkB receptors was recently shown to activate this pathway in oocytes (1). Oocytes from wild-type mice responded to PMSG/hCG stimulation with increased phosphorylation of both AKT (at Ser 473) and the downstream ribosomal protein S6 (at Ser240/244). In contrast, the oocytes from *OoTrkB*^{-/-} mice failed to mount this phosphorylation response. These results suggest that: 1) TrkB-FL receptor expression is induced in oocytes by the preovulatory LH surge, and 2) that activation of newly formed TrkB-FL receptors sets in motion a PI3K/AKT signaling pathway that is essential for oocytes to survive at the onset of adult reproductive cyclicity.

(1)Zhang et al, Theriogenology 2010; 73:1096

Sources of Research Support: NIH Grant HD024870 awarded to SRO.

Nothing to Disclose: MDD, CG-R, BX, GAD, SRO

Pub # OR21-6

Session Information ORAL SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Control of Reproductive Function & Dysfunction (11:15 AM-12:45 PM)

Title Mice Deficient in Surfactant Protein-A (SP-A) and D (SP-D) and the Putative SP-A Receptor TLR2 Exhibit a Significant Delay in Timing of Parturition and Decreased Expression of Contraction-Associated Genes

Author String AP Montalbano, CR Mendelson
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Body Term and preterm labor are associated with an inflammatory response, with increased levels of proinflammatory cytokines in amniotic fluid (AF), infiltration of myometrium, cervix, and fetal membranes by neutrophils and macrophages (M[phi]), and with activation of proinflammatory transcription factors in myometrium. We previously obtained compelling evidence that the fetus provides a critical signal for the initiation of labor near term through developmental induction of surfactant protein (SP)-A expression by the fetal lung and its secretion into AF, where it activates M[phi], triggering their migration to the pregnant uterus. We propose that interactions of M[phi] Toll-like receptors (TLRs) with SP-A, at term, or bacterial lipopolysaccharide at preterm, initiate changes in M[phi] phenotypic properties, resulting in their activation and chemotaxis to the uterus. Due to the potential role of SP-A in the initiation of labor and its structural and functional relatedness to SP-D, in this study, the timing of labor was assessed during first and second pregnancies of WT mice and mice homozygous null for *SP-A*, *SP-D* and for *SP-A/D* genes and in first pregnancies of mice homozygous null for *TLR2*. In first pregnancies, *SP-A*^{-/-}, *SP-D*^{-/-} and *SP-A/D*^{-/-} females bred to genetically like males delivered at term (~19.4 days post-coitum, dpc), whereas *TLR2*^{-/-} females bred to like males manifested a significant (p<0.03) delay in the timing of labor. Interestingly, during second pregnancies, *SP-A* null females bred to genetically-like males manifested a delay, albeit insignificant (p<0.2) in the timing of labor. In contrast, parturition was significantly (p<0.01) delayed in *SP-A/D*^{-/-} females bred to like males. Moreover, myometrial tissues of *SP-A/D*^{-/-} females had significantly decreased mRNA levels for CNX-43 and OXTR and markedly reduced levels of interleukin-1 and -6, as compared to gestation-matched WT mice. Mice deficient in *TLR2* also manifested decreased levels of myometrial CNX-43 mRNA. Together, these findings suggest that the signals leading to the initiation of labor are multifactorial and their relative impacts may be dependent upon parity. Whereas, in the first pregnancy, uterine stretch may serve as a primary and overriding signal, during subsequent pregnancies, due to increased elasticity of the uterus, other signals (e.g. SP-A) may play a more critical role. Our findings further suggest that TLR2 may serve as a critical and common mediator of these diverse signaling pathways.

Sources of Research Support: NIH PO1-HD11149 and MOD grant #21-FY04-174.

Nothing to Disclose: APM, CRM

Pub # OR22-1
Session Information ORAL SESSION: BASIC/TRANSLATIONAL - Genetics & Biochemistry of Steroidogenesis (11:15 AM-12:45 PM)
Title Switching CYP17 Function, from Androgen to Cortisol Synthesis: Insight into Protein-Protein Interactions
Author String LL Martin, S Praporski, CI Yeung, CJ Cobin, N Hatzirodos, RJ Auchus, RJ Rodgers, AJ Conley
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Body Cytochrome P450c17 (CYP17, P450c17) is a steroidogenic enzyme which catalyses both the 17 α -hydroxylation of pregnenolone and progesterone and also subsequent 17,20 lyase reactions. Regulation of these reactions is essential as cortisol synthesis is derived from the first reaction and androgens from the second. Like all P450 enzymes, P450c17 is catalytically inactive without a redox partner binding protein, NADPH-cytochrome P450 oxido-reductase (CPR). Furthermore, we have shown that the protein cytochrome b5 (cyt. b5) promotes the 17,20 lyase reaction,[1] supporting the hypothesis that it functions as an allosteric regulator with the P450c17:CPR complex. Both these reactions are achieved via protein-protein interactions that provide a critical level of control over steroid synthesis. However, to date there are no direct studies of the protein-protein interactions between P450c17 and its' partner proteins and the contribution to the regulation of cortisol and androgen production.

We have used a quartz crystal microbalance (QCM) to [ldquo]weigh[rdquo] proteins as they assemble at a membrane coated chip. Binding [ldquo]dampens[rdquo] the frequency of the resonating quartz crystal as the mass increases (ie. $\Delta m \propto -\Delta f$).[2] Simultaneously, the conformational or structural changes on binding are also assessed by the change in energy dissipation (ΔD). We have created a cellular environment in the QCM chamber with a lipid membrane, deposited onto the chip prior to introduction of purified proteins that bind in a unique way to the phosphatidylcholine: cholesterol (PC:chol) membrane layer. Our QCM results found that the [ldquo]fingerprints[rdquo] for the CPR:P450c17 (binary) and CPR:P450c17:cytb5 (ternary) complex mixtures assembled on a membrane under equilibrium conditions differed. The QCM data also suggest that there are structural differences between the binary and ternary complexes, following assembly at the membrane layer. Our data provide insight to the biophysical mechanisms regulating steroid hormone synthesis via protein-protein interactions with P450c17. The implication for a novel treatment of androgen dependent cancers will be discussed.

1 Naffin-Olivos J. L., Auchus, R. J. Human cytochrome b5 requires residues E48 and E49 to stimulate the 17,20-lyase activity of cytochrome P450c17, *Biochemistry*, 2006, 45, 755-762.

2 Praporski, S., Ng, S., Nguyen, A., Corbin, C.J., Mechler, A., Zheng, J., Conley, A.J., Martin, L.L., Organization of Enzymes Involved in Sex Steroid Synthesis: Protein-protein interactions in lipid layers, *J. Biol. Chem.* (2009), 284(48) 33224-33232.

Nothing to Disclose: LLM, SP, CIY, CJC, NH, RJA, RJR, AJC

Pub # OR22-2

Session Information ORAL SESSION: BASIC/TRANSLATIONAL - Genetics & Biochemistry of Steroidogenesis (11:15 AM-12:45 PM)

Title Mutant Cytochrome B5 Causing 46,XY Disorder of Sex Development (DSD) Due to Apparent CYP17A1 17,20 Lyase Deficiency

Author String J Idkowiak, T Randell, V Dhir, P Patel, CHL Shackleton, N Krone, W Arlt
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Body In humans, androgen synthesis depends on the enzyme CYP17A1 expressed in adrenals and gonads. CYP17A1 has two distinct catalytic activities: its 17 α -hydroxylase activity is crucial for glucocorticoid production in the adrenals. The 17,20 lyase activity of CYP17A1 catalyses the key step in human androgen biosynthesis, the conversion of 17-hydroxypregnenolone to the universal sex steroid precursor dehydroepiandrosterone (DHEA). For catalytic activity, CYP17A1 requires electron transfer from P450 oxidoreductase (POR). Mutations in CYP17A1 and POR have been shown to disrupt human androgen synthesis thereby causing 46,XY DSD. Cytochrome B5 (CYB5) is thought to act as an allosteric facilitator, enhancing the interaction of CYP17A1 and POR proteins, a requirement for normal 17,20 lyase function. We have identified a disease-causing CYB5A missense mutation in a large consanguineous family including three children with 46,XY DSD. The index case, a 46,XY female, presented with lack of pubertal development at 13 years of age. She was fully pre-pubertal and her genitalia appeared female except for mild clitoromegaly; the gonads were not palpable. Urinary steroid profiling by gas-chromatography/mass spectrometry revealed low or undetectable concentrations of the androgen metabolites etiocholanolone and androsterone with elevated excretion of pregnenetriol, the main metabolite of 17-hydroxypregnenolone, an overall profile indicative of isolated 17,20-lyase deficiency. No mutations were found in CYP17A1 or POR. However, we detected a homozygous CYB5 missense mutation replacing highly conserved histidine with leucine at amino acid position 44 (p.H44L). Segregation analysis revealed parental heterozygosity for p.H44L while the two younger brothers presenting with perineal hypospadias and bifid scrotum were homozygously affected. We performed functional in vitro characterisation by co-expressing wild type and mutant CYB5 with CYP17A1 in HEK293 cells, employing a bi-cistronic construct. Kinetic analysis revealed that p.H44L CYB5 decreases 17,20 lyase activity of CYP17A1 to 40 % of wild type activity while 17 α -hydroxylase activity was not affected by mutant CYB5.

We have identified the first human CYB5 missense mutation, a novel co-factor mutation causing androgen deficiency. The considerable phenotypic variability in the degree of male undermasculinization indicates variable penetrance, e.g. due to genetic differences in other determinants of 17,20 lyase activity.

Disclosures: WA: Consultant, Roche Pharmaceuticals. Nothing to Disclose: JI, TR, VD, PP, CHLS, NK

Pub # OR22-3

Session Information ORAL SESSION: BASIC/TRANSLATIONAL - Genetics & Biochemistry of Steroidogenesis (11:15 AM-12:45 PM)

Title Two Pathways of Fetal Testicular Androgen Biosynthesis Are Needed for Male Sexual Differentiation

Author String CE Flueck, AV Pandey, P Kempna, WL Miller, EJ Schoenle, A Biason-Lauber
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Body Human sexual determination is initiated by a cascade of genes that lead to the development of the fetal gonad. Whereas development of the female external genitalia does not require fetal ovarian hormones, male genital development requires the action of testicular testosterone (T) and its more potent derivative, dihydrotestosterone (DHT). The 'classic' biosynthetic pathway from cholesterol to T in the testis and the subsequent conversion of T to DHT in genital skin is well established, and genetic males with disorders in this pathway have underdeveloped external genitalia. Recently, an alternative pathway leading to DHT has been described in marsupials, but its potential importance to human development is unclear. AKR1C2 is an enzyme that participates in the alternative but not the classic pathway. Using a candidate gene approach, we identified AKR1C2 mutations with sex-limited recessive inheritance in three 46, XY individuals with disordered sexual development. Analysis of the inheritance of microsatellite markers excluded other candidate loci. Affected individuals had moderate to severe undervirilization at birth; when recreated by site-directed mutagenesis and expressed in bacteria, the mutant AKR1C2 had diminished, but not absent catalytic activities. 46, XY DSD individuals also carry a mutation causing aberrant splicing in AKR1C4 gene encoding an enzyme with similar activity and distribution. This suggests a digenic mode of inheritance where the severity of the developmental defect depends on the number of mutations in the two genes. These findings suggest that both the classic and alternative pathways of testicular androgen biosynthesis are needed for normal human male sexual differentiation.

Sources of Research Support: Swiss National Science Foundation grants 320000-116299 and 320000-116636

Nothing to Disclose: CEF, AVP, PK, WLM, EJS, AB-L

Pub # OR22-4

Session Information ORAL SESSION: BASIC/TRANSLATIONAL - Genetics & Biochemistry of Steroidogenesis (11:15 AM-12:45 PM)

Title Aromatase Excess Syndrome Caused by Cryptic Duplications and Deletions Leading to Gain-of-Function of *CYP19A1*

Author String M Fukami, M Shozu, S Soneda, F Kato, A Inagaki, H Takagi, K Hanaki, S Kanzaki, K Ohyama, T Sano, T Nishigaki, S Yokoya, G Binder, R Horikawa, T Ogata
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Nagoya Second Red Cross Hospital, Nagoya, Japan; Tottori University, Yonago, Japan; Tottori University, Yonago, Japan; Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, Chuo, Japan; Osaka Police Hospital, Osaka, Japan; National Medical Center for Children and Mothers, Tokyo, Japan; University-Children's Hospital, Tuebingen, Germany

Body
Background: Aromatase is a P450 enzyme that plays a critical role in estrogen biosynthesis. Overexpression of *CYP19A1* encoding aromatase causes a rare autosomal dominant disorder known as aromatase excess syndrome (AEXS) characterized by gynecomastia. Although chromosomal inversions leading to abnormal fusion between *CYP19A1* coding exons and non-coding exons of neighboring genes have been identified in a few patients with AEXS, its molecular basis and clinical spectrum remain largely unknown.
Patients: We studied 18 affected males from families A-F. Blood analyses showed elevated estradiol/testosterone ratios and FSH-dominant hypogonadotropic hypogonadism in all cases examined. RT-PCR and enzymatic assays revealed elevated mRNA levels of *CYP19A1* and increased catalytic activities of aromatase in skin fibroblasts of four cases examined.
Molecular analysis: Comparative genomic hybridization analysis identified a heterozygous genomic rearrangements in all cases; 79,156 bp tandem duplication involving seven of 11 non-coding *CYP19A1* exons 1 in families A and B, a 211,631 bp deletion involving exons 2-43 of *DMXL2* and exons 5-10 of *GLDN* in family C, and a 165,901 bp deletion involving exons 2-43 of *DMXL2* in families D-F. Transcript analysis revealed that the duplications increased the number of functioning physiological *CYP19A1* transcription start sites, and the deletions produced the same chimeric mRNA consisting of *DMXL2* exon 1 and *CYP19A1* coding exons. The *DMXL2* exon 1 harbored a translation start codon, and the *DMXL2/CYP19A1* chimeric mRNA was identified in only 2-5% of *CYP19A1*-positive transcripts. This was contrastive to the inversion-mediated chimeric mRNAs that had no coding sequence on the fused exon 1 and accounted for >80% of *CYP19A1*-positive transcripts. *CYP19A1* was expressed in a limited number of tissues, whereas its neighboring genes involved in the chimeric mRNA formation were expressed widely. Clinical features were milder in patients with duplications/deletions than in those with inversions.
Discussion: The present study shows that AEXS can be caused by duplications of the physiological promoters and deletions of the upstream regions of *CYP19A1*, and that phenotypic severity is primarily determined by the tissue expression pattern of *CYP19A1* and the chimeric genes and by structural properties of the fused exons. Thus, this study provides novel models for the gain-of-function mutations leading to human genetic disease.

Nothing to Disclose: MF, MS, SS, FK, AI, HT, KH, SK, KO, TS, TN, SY, GB, RH, TO

Pub # OR22-5

Session Information ORAL SESSION: BASIC/TRANSLATIONAL - Genetics & Biochemistry of Steroidogenesis (11:15 AM-12:45 PM)

Title Mutations in GPX1 and NNT, Encoding Antioxidant Defense Genes, Cause Familial Glucocorticoid Deficiency

Author String LA Metherell, E Meimaridou, J Kowalczyk, L Guasti, P Nurnberg, NP Mann, R Banerjee, N Saka, P Touraine, XG Lei, PJ King, AJL Clark
WHRI, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, London, UK; University of Cologne, Cologne, Germany; Royal Berkshire Hospital, Reading, UK; NHS Foundation Trust, Luton, UK; University of Istanbul, Istanbul, Turkey; University Pierre et Marie Curie, Paris, France; Cornell University, Ithaca, NY

Body Inherited adrenocorticotropin (ACTH) resistance diseases are rare and include triple A syndrome (OMIM 231550) and familial glucocorticoid deficiency (FGD;OMIM 202200). Clinically, FGD is characterized only by ACTH resistance, while triple A syndrome exhibits additional clinical features including a variety of neurological impairments. Half of FGD cases are caused by mutations in one of three genes; MC2R, MRAP or STAR, all genes in the steroidogenic pathway. Mutations in AAAS encoding ALADIN are responsible for the majority of Triple A cases.
SNP array genotyping of FGD patients mapped disease loci to chromosome 3p26-24 and 5p13-q12. Targeted exome sequencing of 5p13-q12 in one patient identified a homozygous mutation, p.Ala533Val, in nicotinamide nucleotide transhydrogenase (NNT), a protein involved in antioxidant defence. Further mutations, p.Gly600ValfsX7 and p.Leu977Pro, were discovered by conventional sequencing in two other families with linkage to this locus. The antioxidant gene glutathione peroxidase 1 (GPX1) was subsequently identified within the linked region on chromosome 3 and sequencing detected a homozygous mutation, p.Arg130-Leu133del, in one individual.
RT-PCR revealed that NNT and GPX1 are both highly expressed in human adrenals. Adrenal histology in C57BL6/J (a spontaneous NNT mutant) and GPX1 KO mice showed grossly normal morphology with some evidence of adrenal hyperplasia and capsular thinning. Interestingly, an autopsy on a 5 month old sibling of the GPX1 mutant reported ectopic adrenal glands displaying loss of the zona fasciculata with relative preservation of the zona glomerulosa.
NNT encodes an integral protein of the inner mitochondrial membrane. Under most physiological conditions, this enzyme uses energy from the mitochondrial proton gradient to produce high concentrations of NADPH. The resulting NADPH is used for biosynthesis and in ROS detoxification by enzymes such as GPX1. Previous studies have shown that ROS-mediated disruption of Leydig cell mitochondria inhibits STAR function and Leydig cell steroidogenesis, and recent work implicates oxidative stress in the pathogenesis of triple A syndrome. Our results suggest that NNT and GPX1 are critical enzymes for ROS detoxification in adrenocortical cells, with their loss leading to defective oxidative stress responses, an impairment of steroidogenesis and hence adrenal insensitivity to ACTH.

Sources of Research Support: Medical Research Council United Kingdom, New Investigator Research Grant G0801265 awarded to LAM.

Nothing to Disclose: LAM, EM, JK, LG, PN, NPM, RB, NS, PT, XGL, PJK, AJLC

Pub # OR22-6

Session Information ORAL SESSION: BASIC/TRANSLATIONAL - Genetics & Biochemistry of Steroidogenesis (11:15 AM-12:45 PM)

Title Minichromosome Maintenance Complex Component 4 (*MCM4*) Mutation Is Associated with a Unique Form of Adrenal Failure Found in the Irish Traveller Community and Is Implicated in Natural Killer Cell Deficiency and Increased Chromosome Fragility

Author String CR Hughes, E Meimaridou, L Guasti, C-h Chuang, JC Schimenti, PJ King, C Costigan, AJ Clark, LA Metherell
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Body A unique variant of Familial Glucocorticoid Deficiency (FGD) exists in the Irish travelling community, a genetically isolated population with high levels of consanguinity. Affected children develop hypocortisolaemia and raised ACTH but retain normal renin and aldosterone levels. Children have short stature, evidence of increased chromosomal breakage and natural killer (NK) cell deficiency. Mutations in *MC2R*, *MRAP* and *STAR* have been excluded.
SNP genotyping of 5 affected individuals identified 3 common areas of homozygosity. Targeted exome sequencing identified one variant (c.71-1insG) in minichromosome maintenance complex component 4 (*MCM4*), which was homozygous in 8 affected patients, heterozygous in parents and absent in controls. RT-PCR of patient leukocyte RNA revealed this change leads to incorporation of an extra G (r.70_71insg) resulting in a foreshortened ORF encoding a prematurely terminated translation product (p. Pro24ArgfsX4). *MCM4* is one of 6 homologous MCM proteins (*MCM2-7*) that form a heterohexameric complex essential for normal DNA replication and genome stability in all eukaryotes. MCMs are abundantly expressed in proliferating cells and are vital to survival; *MCM KO* in mice is lethal and no *MCM* mutation has ever been described in humans.
Mice with one disrupted *MCM4* allele and one hypomorphic *MCM4* allele (*Chaos 3*; F345I), demonstrate embryonic or neonatal lethality however additional *MCM3* heterozygosity significantly increases viability. Histological examination of the adrenals of *MCM4*^{Chaos3/-} *MCM3*^{+/-} mice, the closest viable animal model to our patient cohort, revealed an abnormal adrenal morphology with small, spindle-shaped, CYP11A1 negative, GATA-4 positive cells infiltrating the cortex, resulting in a reduced number of steroidogenic cells in the zona fasciculata. This morphology is not seen in *MCM4*^{+/+} *MCM3*^{+/-} mice.
Deficiency of *MCM4* causes DNA replication defects and is therefore a likely cause of the increased chromosomal fragility in these patients. This can also lead to apoptosis and we have shown that the zona fasciculata cells in the mutant mice expressed a marker of apoptosis (Cleaved-caspase 3) at higher levels than wild type. In conclusion we have identified the first human mutation in *MCM4* in a cohort of patients with a unique form of late onset adrenal failure associated with NK cell deficiency and increased chromosome breakage. Mice harbouring a similar mutation have grossly abnormal adrenals.

Sources of Research Support: Medical Research Council UK; European Society Paediatric Endocrinology Research Fellowship.

Nothing to Disclose: CRH, EM, LG, C-HC, JCS, PJK, CC, AJC, LAM

Pub # OR23-1
Session Information ORAL SESSION: BASIC/TRANSLATIONAL - Molecular Mechanisms Regulating Glucose Homeostasis & Insulin Action (11:15 AM-12:45 PM)

Title Central Regulation of Endogenous Glucose Production (EGP) Is Impaired in Type 2 Diabetes Mellitus (T2DM)

Author String D Mehta, S Bursheh, W Li, K Zhang, L Boucai, P Kishore, M Hawkins
Albert Einstein College of Medicine, Bronx, NY

Body Endogenous glucose production (EGP) is increased in T2DM, indicating loss of normal regulation by insulin and glucose. Rodent studies suggest that these factors suppress EGP at least in part through activation of hypothalamic K_{ATP} channels, and their suppressive effects on EGP are reproduced by intracerebroventricular (ICV) administration of diazoxide (DZX). Of note, we demonstrated that oral DZX reduced EGP by ~66% in nondiabetic subjects under fixed hormonal conditions (1).
To verify that orally administered DZX regulates EGP via central K_{ATP} channels, we studied the effects of DZX vs. saline gavage in n=17 normal male Sprague Dawley rats. EGP ($3\text{-}^3\text{H}$ -glucose) was quantified from 4 to 6 hours post-gavage under euglycemic 'pancreatic' (somatostatin, basal insulin) clamp conditions, to exclude known effects of DZX on pancreatic K_{ATP} channels. DZX concentrations in cerebrospinal fluid were comparable to prior ICV studies (0.76 ± 0.2 [$\mu\text{g}/\text{ml}$]). Oral DZX significantly decreased EGP (DZX = 2.20 ± 0.28 vs. Plc = 4.85 ± 0.40 mg/kg.min; 55% suppression, $p < 0.0001$), increased hepatic STAT-3 phosphorylation (40% increase, $p = 0.001$) and reduced hepatic expression of PEPCK (30% decrease, $p = 0.012$) and Glucose-6-phosphatase (72% decrease, $p = 0.028$). These effects were all negated by ICV administration of the K_{ATP} channel blocker glibenclamide, suggesting that oral DZX exerted its effects on EGP by activating hypothalamic K_{ATP} channels.
We next examined whether these central mechanisms of regulation of EGP might be impaired in humans with T2DM. Paired euglycemic, 4 hour pancreatic clamp studies were performed 3 hours after oral diazoxide (6 mg/kg) vs. placebo (Plc) administration in randomized, double blind fashion to n=8 otherwise healthy individuals with T2DM (5 males; age 49 ± 2 years, BMI 32 ± 1 kg/m², HbA1c $9.4 \pm 0.6\%$). Somatostatin was infused along with glucagon/growth hormone replacement, with maintenance of basal insulin (~ 20 [$\mu\text{U}/\text{ml}$]) and glucose (~ 90 mg/dl) concentrations throughout. Under fixed hormonal conditions, oral diazoxide failed to suppress EGP in these poorly controlled T2DM individuals (DZX = 1.47 ± 0.08 versus Plc = 1.65 ± 0.07 mg/kg.min, $p = 0.21$).
Together, these data suggest that orally administered diazoxide exerts central effects on glucose production in humans, and this regulation is impaired in poorly controlled T2DM. Chronic upregulation of hepatic glucose fluxes in T2DM might impair the ability of central mechanisms to suppress EGP.

(1) Boucai L et al, Diabetes 2008;57(supplement 1):1497P

Sources of Research Support: DK 069861 and the American Diabetes Association.

Nothing to Disclose: DM, SB, WL, KZ, LB, PK, MH

Pub # OR23-2

Session Information ORAL SESSION: BASIC/TRANSLATIONAL - Molecular Mechanisms Regulating Glucose Homeostasis & Insulin Action (11:15 AM-12:45 PM)

Title Importance of the β -Cell Androgen Receptor in Type 2 Diabetes

Author String G Navarro, S Liu, K De Gendt, G Verhoeven, F Mauvais-Jarvis
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Body In women, androgen excess predisposes to insulin resistance and type 2 diabetes (T2D). Still, limited attention has been devoted to the role of androgen excess in β -cell dysfunction. In aging men, androgen deprivation contributes to the development of the metabolic syndrome and T2D, but the role of androgen deficiency in β -cell dysfunction is unknown. Furthermore, there is no information on the role of the androgen receptor (AR) in β -cells in physiology and diabetes. To address the role of the AR in β -cells in vivo, we generated a β -cell-specific AR deficient mouse (β ARKO $-/-$) using the Cre-loxP strategy. β ARKO $-/-$ mice showed selective loss in islet AR gene and protein expression without alteration in AR expression in other tissues including the hypothalamus. Female mice were exposed to excess androgen using the AR-agonist dihydrotestosterone (DHT). On a normal chow, hyperandrogenic female and male control and β ARKO $-/-$ mice showed no significant alteration in β -cell function and glucose homeostasis. Mice were exposed to a metabolic stress induced by a high fat diet (HFD). Following HFD challenge, hyperandrogenic female control mice developed islet abnormalities characteristic of early T2D with compensatory β -cell hyperplasia, increased islet insulin content, hyperinsulinemia and β -cell failure to compensate for insulin resistance. These mice developed hyperglycemia in the fasting and fed state and following a glucose challenge. Conversely, hyperandrogenic β ARKO $-/-$ mice did not develop overt islet abnormalities and remained normoglycemic. Furthermore, β ARKO $-/-$ male mice exposed to a HFD became hyperglycemic following a glucose challenge compared to control littermates. In conclusion, on a western HFD environment, excess AR activation in β -cells of females or loss of AR activation in β -cells of males causes β -cell failure and predisposes to T2D. Funded by NIH grants DK074970, HD044405.

Nothing to Disclose: GN, SL, KDG, GV, FM-J

Pub # OR23-3
Session Information ORAL SESSION: BASIC/TRANSLATIONAL - Molecular Mechanisms Regulating Glucose Homeostasis & Insulin Action (11:15 AM-12:45 PM)
Title Identification of a Novel Phosphatase Regulating Glucose Metabolism through Its Expression in Osteoblasts
Author String T Zee, C Settembre, G Karsenty
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Body Osteocalcin is a bone-derived hormone necessary for whole body glucose homeostasis that is activated by insulin signaling in the osteoblast (1). Once activated, osteocalcin binds to a specific receptor on beta cells of the pancreas to promote insulin secretion, and on other cell types to improve insulin sensitivity and energy expenditure (2). Within the osteoblasts, an important negative regulator of insulin signaling, and therefore of osteocalcin activity, is OST-PTP, a protein tyrosine phosphatase (PTP) whose substrate is the insulin receptor (1). In this study we sought to identify additional PTPs present in the osteoblast that would dephosphorylate the insulin receptor and thus, be important for activating osteocalcin. Our rationale was two-fold. First, bone, like other insulin-responsive tissues, may use multiple PTPs to attenuate the insulin signal. Secondly, *Esp*, the gene encoding OST-PTP, is a pseudogene in human, yet genetic evidence indicates that the insulin-osteocalcin activation pathway is conserved in humans (1). Hence, other PTPs must exist in osteoblast and regulate insulin signaling in this cell. We focused our attention on the tyrosine phosphatase TC-PTP, because it is expressed in both mouse and human osteoblasts and is able, *in vitro*, to interact with the insulin receptor. We show here through multiple assays that the insulin receptor is a substrate of TC-PTP in osteoblasts. Accordingly, insulin receptor phosphorylation and insulin target gene expression are amplified in osteoblasts lacking this phosphatase. To more firmly establish the biological importance of TC-PTP expression in osteoblasts *in vivo*, we performed a cell-specific gene inactivation in the mouse. Consistent with our hypothesis, mice lacking TC-PTP specifically in osteoblasts display an increase in serum levels of the active form of osteocalcin. As a result these mutant mice develop a marked increase in insulin sensitivity, indicating that TC-PTP is a significant player in the regulation of energy metabolism exerted by bone. A full characterization of the function exerted by TC-PTP in the regulation of glucose metabolism through its expression in osteoblasts will be presented at the meeting.

(1) Ferron M et al., Cell 2010; 142:2

(2) Lee NK et al., Cell 2007; 130:3

Sources of Research Support: Hormones: Biochemistry and Molecular Biology - T32DK07328.

Nothing to Disclose: TZ, CS, GK

Pub # OR23-4

Session Information ORAL SESSION: BASIC/TRANSLATIONAL - Molecular Mechanisms Regulating Glucose Homeostasis & Insulin Action (11:15 AM-12:45 PM)

Title Lack of a Beneficial Metabolic Profile in Liver-Specific 11 β -Hydroxysteroid Dehydrogenase Type 1 (11 β -HSD1) Knockout Mice

Author String GG Lavery, LL Gathercole, SA Morgan, A Zielinska, K Saqib, B Hughes, EA Walker, PM Stewart
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Body In humans glucocorticoid (GC) excess can promote hepatic glucose and triglyceride production contributing to obesity, fatty liver and diabetes. 11 β -HSD1 activates GCs (11-DHC to corticosterone in mice), thereby increasing tissue concentrations. The liver has the highest 11 β -HSD1 activity, and its inhibition has been proposed as a therapeutic option to treat diabetes through reduction of hepatic glucose output. To investigate this we generated 11 β -HSD1 liver-specific knockouts (HSD1LKO) and examined GC metabolism and responses to high-fat diet (HFD).
We generated HSD1LKOs using an albumin-Cre transgenic mouse and showed abolished 11 β -HSD1 activity only in the liver; global 11 β -HSD1 knockout (HSD1KO) mice were used as additional controls.
An oral cortisone challenge resulted in serum cortisol concentrations of 1830 \pm 184nM (control), 691 \pm 70nM (HSD1LKO) and 67 \pm 24nM (HSD1KO) (control vs. HSD1LKO p<0.002, n=7-9), indicating significant extra-hepatic GC production in HSD1LKOs. Corticosterone/11-DHC urinary metabolite ratios were the same in HSD1LKO and controls (0.08) and elevated (0.5) in HSD1KOs, indicating that despite diminished hepatic GC production there is no impact on hepatic metabolism set-point in HSD1LKOs.
HSD1LKO and controls had similar basal serum corticosterone concentrations and adrenals were only significantly larger in HSD1KOs (n=9-11). HSD1LKO and controls had similar body, liver, epididymal fat pad and muscle weight (n=9-11). No differences were observed for fed and fasting glucose on regular and HFD. HSD1LKO mice were mildly protected from glucose intolerance on HFD when subjected to a glucose tolerance test but not to the same degree as HSD1KO mice (n=7-9). Both HSD1LKO and controls developed fatty liver on HFD assessed by Oil Red O staining and hepatic triglyceride (TAG) assays, and no differences were observed between serum TAG, NEFA and cholesterol profiles (n=6-7).
The lack of a major effect upon cortisone/ 11DHC metabolism in the HSD1LKO suggests that the liver is not, as widely believed, the principal site of 11 β -HSD1-mediated metabolism. HSD1LKOs have little protection from the metabolic consequences of a HFD as seen in HSD1KOs. This model produces a novel insight into the target for 11 β -HSD1 inhibition with extra-hepatic enzyme expression playing an important role.

Sources of Research Support: BBSRC and the Wellcome Trust.

Nothing to Disclose: GGL, LLG, SAM, AZ, KS, BH, EAW, PMS

Pub # OR23-5

Session Information ORAL SESSION: BASIC/TRANSLATIONAL - Molecular Mechanisms Regulating Glucose Homeostasis & Insulin Action (11:15 AM-12:45 PM)

Title The Molecular Mechanism of IRS-1 Serine Phosphorylation-Mediated Insulin Resistance

Author String JR Woo, SY Park, TE Wales, JR Engen, SE Shoelson
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Body Serine and threonine phosphorylation of IRS-1 by JNK and a host of other Ser/Thr kinases is widely thought to be a molecular mechanism for obesity-, stress- and inflammation-induced insulin resistance and type 2 diabetes. While phosphorylation at Ser307 (in mice; Ser312 in humans) is necessary for inhibition, it is not sufficient. Additional phosphorylation sites are also required, including Ser302 and others in the vicinity. An extensive series of structure-function studies now show that a region of IRS-1, C-terminal to its PH (residues 12-116) and PTB (residues 160-264) domains and encompassing numerous serine phosphorylation sites including Ser302 and Ser307, creates a newly identified domain that binds the insulin receptor when it is not serine-phosphorylated. By contrast, when the phosphorylation insulin resistance (PIR) domain of IRS-1 it is serine-phosphorylated it does not bind the insulin receptor. BIAcore studies show that the PTB and PIR domains bind the insulin receptor kinase (IRK) independently. However, when both domains are present binding is cooperative -- the PTB-PIR region of IRS-1 binds IR with 40-fold higher affinity than the PTB domain alone. PIR phosphorylation at Ser302, Ser307, Ser310 and/or Ser318 abolished both independent PIR domain binding and cooperative PTB-PIR domain binding. Hydrogen-deuterium exchange mass spectrometry showed that the PIR domain binds the amino-terminal lobe and hinge regions of the insulin receptor kinase. IRK assays showed that PIR domain binding does not affect tyrosine phosphorylation of insulin receptor substrates. However, PIR domain binding does protect IRK from phosphotyrosine phosphatase (PTP) mediated dephosphorylation. Thus PIR enhances insulin signaling by at least two potential mechanisms: 1) direct binding to IRK to facilitate multisite tyrosine phosphorylation along with the PTB domain, and 2) inhibition of IRK tyrosine dephosphorylation. Serine phosphorylation of PIR abolished both effects, which causes insulin resistance. In summary we have identified a new domain in IRS-1 that binds IRK to enhance insulin signaling. Serine phosphorylation of PIR abrogates binding to cause insulin resistance.

Sources of Research Support: NIH R01 DK43123.

Nothing to Disclose: JRW, SYP, TEW, JRE, SES

Pub # OR23-6

Session Information ORAL SESSION: BASIC/TRANSLATIONAL - Molecular Mechanisms Regulating Glucose Homeostasis & Insulin Action (11:15 AM-12:45 PM)

Title GQ-16: A Novel Partial PPAR γ Agonist with Potent Insulin-Sensitizing Activity without Inducing Weight Gain, and a Distinct Binding Mode to PPAR γ LBD

Author String FdAR Neves, S Rajagopalan, BM Carvalho, MJA Saad, M Togashi, LA Simeoni, ACM Figueira, I Polikarpov, MDCA Lima, SL Galdino, IdR Pitta, P Webb, S Ayers, K Phillips, AA Amato
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Body The effectiveness of thiazolidinediones (TZDs) such as rosiglitazone (RSG) and pioglitazone (PIO) for treatment of type 2 diabetes has been validated extensively in humans. TZDs act through peroxisome proliferator-activated receptor gamma (PPAR γ) to improve insulin sensitivity, but trigger undesirable side effects such as weight gain, congestive heart failure and bone loss. Clinical studies indicate that RSG increases mortality from heart attack relative PIO. Recent data, however, have raised concerns about the cardiovascular safety of PIO, and underscore the need for new selective PPAR γ modulators (SPPARMs) with improved safety profiles. In this study, we describe a novel synthetic benzylidene-TZD (GQ-16; [5-(5-bromo-2-methoxy-benzylidene)-3-(4-methyl-benzyl)-thiazolidine-2,4-dione]) that acts a potent anti-diabetic and insulin sensitizing agent in Swiss mice with obesity and diabetes induced by high fat diet. GQ-16 displayed weak PPAR γ partial agonism in U-937 promonocytes, and low adipogenic activity in C3H10T1/2 mesenchymal cells. Competition binding assays showed GQ-16 bound directly to the ligand binding domain (LBD) of PPAR γ , albeit with lower affinity than RSG. GQ-16 significantly reduced H/D exchange in helix 12, similarly to rosiglitazone, which contains the ligand-binding domain comprising part of the AF-2 surface of the receptor responsible for agonism. Interestingly, GQ-16 had a more marked impact on H/D exchange kinetics across H3 (amino acids 251-281). This region includes Ser 273, and the phosphorylation of this residue has been shown to dysregulate a large number of genes such insulin-sensitizing adiponectin. X-ray structural analysis revealed that GQ-16 occupies the classic PPAR γ ligand binding pocket (LBP), yet displayed a distinct binding mode from RSG and other TZDs. Analysis of distinctions between GQ-16 and TZD contacts with LBP amino acids and comparisons of the effects of both ligands on PPAR γ conformation and activities suggests likely explanations for its unique activity profile relative to RSG and may indicate a new general strategy for development of SPPARMs that retain anti-diabetic actions of TZDs but lack agonist activities that may underlie harmful side effects.

Nothing to Disclose: FdARN, SR, BMC, MJAS, MT, LAS, ACMF, IP, MdCAL, SLG, IdRP, PW, SA, KP, AAA

Pub # OR24-1

Session Information ORAL SESSION: TRANSLATIONAL - Iodine & Clinical Thyroid Disease (11:15 AM-12:45 PM)

Title 2010 Estimate for the Global Burden of Disease Due to Iodine Deficiency

Author String MB Zimmermann, V Karumbunathan, M Andersson
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Body Deficiencies of micronutrients such as iodine contribute to the global burden of disease (GBD) and interventions to prevent these deficiencies are highly cost-effective strategies for public health action. A decade has passed since the last estimate of the GBD due to iodine deficiency (ID). Because of progress in the control of ID worldwide and the development of new assessment methods, the Child Health Epidemiology Reference Group (CHERG) working with WHO has reassessed the contribution of ID to the GBD. First, a systematic literature search on the effects of ID was done. Then, using these data, a new ID disease model was defined. It included the GBD 1990 and 2000 sequelae of goiter and cretinism, but added sequelae such as hyperthyroidism and borderline, mild, and moderate intellectual impairment. The model was refined by including three levels of ID severity as defined by the WHO criteria for the median urinary iodine concentration (UIC): mild ID, median UIC 50-99 mcg/L; moderate ID, median UIC 20-49 mcg/L and severe ID, median UIC 0-19 mcg/L. Then to accurately define the proportion of the global and regional population affected by these ID levels, the WHO Vitamin and Mineral Nutrition Information System (VMNIS) global database was thoroughly reviewed and updated to produce new global and regional estimates of the prevalence of ID in 2010. The current estimates show that the iodine status worldwide has improved over the last decades. A unique and novel approach was used to quantify the loss of global IQ points associated with varying degrees of ID severity based on the population shift in the IQ distribution. This is extrapolated to the normal distribution of IQ, adjusted for developing regions, and this quantifies the population in which ID results in an IQ shift into the defined ranges of borderline and mild intellectual impairment. Taken together, these new approaches should improve the accuracy of the current estimate of the GBD due to ID and thereby better inform public policy in regional and national health programs to prevent ID.

Sources of Research Support: CHERG, Unicef, ETH Zürich.

Nothing to Disclose: MBZ, VK, MA

Pub # OR24-2

Session Information ORAL SESSION: TRANSLATIONAL - Iodine & Clinical Thyroid Disease (11:15 AM-12:45 PM)

Title PTTG-Binding Factor (PBF) Induces Goitrogenesis and Represses Iodide Uptake [italicIn Vivo

Author String ML Read, GD Lewy, N Sharma, JCW Fong, VE Smith, GA Ryan, RI Seed, PPK Kwan, WE Leadbeater, A Warfield, MC Eggo, JC Watkinson, JA Franklyn, K Boelaert, CJ McCabe
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Body Goitrogenesis is common and its pathogenesis remains poorly understood. Previously, we showed that thyroid-targeted overexpression of the PTTG Binding Factor (PBF) induced significant thyroid gland enlargement in transgenic mice (PBF-Tg) (1). To better understand the role of PBF in goitrogenesis we have now examined thyroid morphology, gene expression and iodide uptake in PBF-Tg and human thyroids. Histological examination showed that PBF-Tg mice were prone to macrofollicular lesions with >90% of mice demonstrating follicles >250 microns in diameter by 18 months of age ($p=0.02$ compared with wild-type (WT) littermates). Focal and nodular hyperplasia was also apparent, with a high occurrence of hyperplastic lesions (>75%) in PBF-Tg mice ($p=0.009$ compared with WT). Sodium iodide symporter (NIS) mRNA was reduced by ~50% ($p=0.0004$) in PBF-Tg thyroids. Critically, iodide uptake was significantly repressed (~70% reduction, $p=0.0004$) in primary thyroid cultures from PBF-Tg mice, which could be rescued by siRNA depletion of PBF. Western blot analysis also showed significant upregulation of phosphorylated Akt (3.4 ± 1.2 -fold, $p=0.0007$) and the TSH receptor (2.6 ± 0.4 -fold, $p=0.02$) in PBF-Tg thyroids, known regulators of thyroid cell proliferation. In support of this, positive immunostaining with the downstream proliferative marker cyclin D1 in diffuse goiter regions was significantly higher in PBF-Tg mice ($n=4834/35958$ positive cells) compared to WT ($n=1231/25987$ positive cells; $p<0.0001$). Further, hyperplastic lesions of PBF-Tg thyroids demonstrated much greater cyclin D1 staining, ranging from 19% to 68% of cells ($37.7\pm 20.7\%$). Finally, we examined PBF and NIS expression in human multinodular goiters (MNG). PBF mRNA expression was up to 5.6-fold higher in MNG than in normal thyroid ($p=0.008$), whereas NIS mRNA expression was significantly reduced by 38% ($p=0.002$). We also found a significant negative correlation between PBF and NIS mRNA expression in MNG ($r_s=0.39$, $p=0.05$, $n=24$). Further, siRNA repression of PBF in human primary thyroid cultures resulted in increased NIS expression (2.1 ± 0.4 -fold, $p=0.02$) and iodide uptake (2.0 ± 0.14 -fold, $p=0.0001$). Altogether, these results demonstrate that PBF overexpression results in increased thyroid cell proliferation, and repressed iodide uptake, the central therapeutic option for thyroid hyperplasia. Given that PBF is overexpressed in human MNG, PBF should now be considered a central gene in common thyroid disorders.

(1) Read ML et al., Endocrine Abstracts 2009; 19:P371

Sources of Research Support: Grants from the Get-Ahead Charity and the Medical Research Council.

Nothing to Disclose: MLR, GDL, NS, JCWF, VES, GAR, RIS, PPKK, WEL, AW, MCE, JCW, JAF, KB, CJM

Pub # OR24-3

Session Information ORAL SESSION: TRANSLATIONAL - Iodine & Clinical Thyroid Disease (11:15 AM-12:45 PM)

Title Dual Oxidase 2 Genetic Modifications in Children with Hypothyroidism: Identification and Functional Analysis of New Variants and Mutations

Author String A Molinaro, G De Marco, L Montanelli, P Agretti, B Bagattini, A Dimida, E Ferrarini, C Di Cosmo, F Niccolai, C Ceccarelli, F Brozzi, A Pinchera, P Vitti, M Tonacchera
University of Pisa, Pisa, Italy

Body Objective: To identify DUOX2 mutations in children with CH or isolated hyperthyreotropinemia and a eutopic thyroid gland. Patients: 21 children with CH and a eutopic thyroid gland and 5 children with isolated hyperthyreotropinemia. In all the children LT4 was stopped to verify thyroid function when they were 3 yo. In 7 children a partial and in 2 children a complete organification defect was shown after 123-I scintigraphy and perchlorate test. In children with the organification defect TPO, DUOX2, DUOXA2 genes were analyzed while in the others DUOX2, DUOXA2, PAX8 and TSH receptor genes were studied. The functional activity of the DUOX2 variants was studied after expression in eukaryotic cells. Mutated DUOX2 proteins were obtained by site-directed mutagenesis. HeLa cells were seeded and co-transfected with the wtDUOX2, DUOX2 mutants or the empty vector in the presence of DUOXA2. After transfection the H2O2 was determined. DUOX2 expression was also determined in transfected HeLa cells by on chip flow cytometry using Agilent 2100 bioanalyzer. Results: No TPO mutations were identified. Direct sequencing of the DUOX2 gene revealed a monoallelic deletion S965FsX994 in three children. Four children showed H678R, R701Q, P983A heterozygous mutations. One child was compound heterozygous for S911L and C1052Y substitutions. Three children were heterozygous for one of the following mutations: A728T, A297S, Y1150C. The functional studies showed the following results: H678R, R701Q and P982A mutants showed the same activity of wtDUOX2 in terms of H2O2 released from the cells. Mutants S965fsX994, A728T, A297S and Y1150C determined an almost complete inhibition of the H2O2 generation in HeLa cells. Mutants S911L and C1052Y determined a milder but significant decrease in H2O2 production in HeLa cells with respect to the wtDUOX2. Cytofluorimetric analysis showed an expression at the cell surface of H678R, R701Q and P982A mutants similar to that of wtDUOX2, while S965fsX994, S911L and C1052Y mutants were significantly less expressed with respect to wtDUOX2. Conclusions: In 26 children with CH or isolated hyperthyreotropinemia and a eutopic thyroid we found 6 mutants (2 in children with isolated hyperthyreotropinemia and 4 in children with CH) of the DUOX2 gene (S965fsX994, A728T, A297S, Y1150C, S911L and C1052Y) determining a reduction in H2O2 production when expressed in vitro, while other 3 gene variants (H678R, R701Q and P982A) were identified in children with CH.

Nothing to Disclose: AM, GDM, LM, PA, BB, AD, EF, CDC, FN, CC, FB, AP, PV, MT

Pub # OR24-4

Session Information ORAL SESSION: TRANSLATIONAL - Iodine & Clinical Thyroid Disease (11:15 AM-12:45 PM)

Title Subtle Health Impairment and Socio-Educational Achievement in Young Adult Patients with Congenital Hypothyroidism Diagnosed by Neonatal Screening: A Longitudinal Population-Based Cohort Study

Author String J Leger, E Ecosse, M Roussey, J-L Lanoe, B Larroque
Hopital Robert Debré, Paris, France; AFDPHE, Paris, France; U687, Villejuif, France; Hopital Beaujon, Paris France

Body Neonatal screening programs, which have been run over the last 30 years in most industrialized countries, have resulted in the prevention of severe cerebral damage and a large decrease in morbidity in patients with congenital hypothyroidism. Previous studies have indicated some difficulties in fine motor area, visuospatial attention, memory and psycho-educational outcome persisting into adolescence and young adulthood. Only a few small studies have examined educational achievement and health-related quality of life (HRQoL), and their conclusions remain unclear.

The aim of this study was to describe self-reported health status, socioeconomic achievement and HRQoL and their determinants, for a population-based registry of young adult patients, compared with those of a reference population of the same age group.

Patients and methods: All 1748 eligible patients diagnosed during the first decade after the introduction of neonatal screening in France and followed in an observational study were invited to participate in this study at a mean age of 23 ± 3 years. Completed questionnaires were obtained from 1202 of the selected patients (69% of the initial cohort). The comparison group included 5817 subjects of the same age, from a national Health Survey.

Results Patients with CH were significantly more likely than their peers to report associated chronic diseases (5.7 vs 2.9%), hearing impairment (9.5 vs 2.5%), visual problems (55.4 vs 47.9%) and being overweight, with a BMI ≥ 25 (22.8 vs 15.7%) ($p < 0.0001$). Furthermore, fewer patients achieved the highest socioeconomic category (14.6 vs 23.1%) and were in full-time employment (39.9 vs 44.8%) ($p < 0.0001$). They were more likely to be still living with their parents and had a lower HRQoL than their healthy peers, particularly for mental dimensions, with a mean difference for the mental summary component of 0.35 SDS ($p < 0.0001$). CH severity at diagnosis, treatment adequacy and the presence of other chronic health conditions were the main determinants of educational achievement and HRQoL scores.

In Conclusion, these findings highlight the need for appropriate attention particularly for patients with severe forms of CH and to monitor the neurosensory functioning and weight of the patients. Careful follow-up of the adequacy of treatment is required to reduce the risk of poor QOL and educational disadvantages. Nonetheless, most patients integrate well into society.

On behalf of the French Congenital Hypothyroidism Study Group.

Sources of Research Support: French Ministry of Health (PHRC) and the Wyeth Fondation.

Nothing to Disclose: JL, EE, MR, J-LL, BL

Pub # OR24-5

Session Information ORAL SESSION: TRANSLATIONAL - Iodine & Clinical Thyroid Disease (11:15 AM-12:45 PM)

Title Time Course to Resolution of Consumptive Hypothyroidism Associated with Infantile Hepatic Hemangioma Treated with Propranolol

Author String JE Emerick, K Hack, NS Larson, KE Jensen, A Bauer
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Body **Background:** Infantile hepatic hemangiomas (IHH) are the most common benign liver tumors of infancy. Diffuse IHH are a less common, but more severe subtype. In 2008 a report reviewing the involution of facial hemangiomas secondary to propranolol therapy was published.(1) Since this time, multiple reports have shown similar effects of propranolol on other hemangiomas, including IHH. IHH may be complicated by severe consumptive hypothyroidism (CH) secondary to overproduction of type III deiodinase (D3) by tumor endothelium. Recently, three patients with IHH and associated CH were described to have resolution of their hypothyroidism within 2-4 weeks of initiating propranolol, coincident with a decrease in tumor size.(2) Herein we report a markedly different clinical course with resolution of CH significantly lagging behind decreased liver size.
Clinical Case: A 10 week old presented with constipation, abdominal distension, and marked hepatomegaly. Large, hypoechoic liver masses with distended vessels surrounding the tumors were found on ultrasound and subsequent triphasic CT confirmed the diagnosis of diffuse IHH. Screening Laboratory evaluation revealed a TSH of 52.5 (normal 0.35-5.5 uIU/mL). The infant was started on prednisolone (2mg/kg/day) and levothyroxine (75mcg/day). Within 48 hours the patient's clinical course worsened and propranolol therapy was initiated (2 mg/kg/day). The patient responded rapidly with improved hemodynamic status and a rapid decrease in liver size. Within 4 weeks TSH normalized on 150mcg of daily levothyroxine. Five months after initiation of propranolol, levothyroxine requirements began to decrease. By 13 months of age levothyroxine could be discontinued. Serial abdominal ultrasounds revealed progressive reduction in liver size, however at 19 months of age persistent diffuse hemangiomas remain.
Conclusion: This case reviews the resolution of consumptive hypothyroidism in response to propranolol therapy for IHH. Propranolol may act via multiple mechanisms, to include inducing vasoconstriction, inhibition of MAPK dependent expression of VEGF and bFGF, and decreased TGF- β induced Smad-dependent production of D3. Thus, propranolol may lead to rapid reduction in tumor size, but as this case highlights, changes in D3 activity may not directly correlate with changes in tumor size and should be followed independently to ensure that patients are not over or undertreated for their CH.

- (1) Léauté-Labr[grave]ze C et al., N Eng J Med. 2008;358(24):2649-51.
(2) Mazereeuw-Hautier J et al., The Journal of Pediatrics 2010; 157:340-2.

Nothing to Disclose: JEE, KH, NSL, KEJ, AB

Pub # OR24-6

Session Information ORAL SESSION: TRANSLATIONAL - Iodine & Clinical Thyroid Disease (11:15 AM-12:45 PM)

Title Development of an Objective Tool for the Diagnosis of Myxedema Coma

Author String YV Chiong, CN Mariash
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Body Myxedema coma (MC) is rare entity that often presents as a diagnostic challenge. If unrecognized and untreated, it is often fatal with mortality reported as high as 30-50% due, in part, to various recommendations for treatment. To date there have been no reported objective criteria to aid with making the diagnosis. Therefore, we performed a retrospective chart review of patients admitted with the diagnosis of MC and developed an objective screening tool to help correctly classify such patients. All patients aged 18 years or older admitted to Methodist Hospital between January 1, 2005 to June 13, 2010 with a diagnosis of MC were identified. Heart rate, temperature and Glasgow Coma Scale (GCS) were measured at the time of admission. TSH and free T4 (FT4) when measured during the hospitalization were recorded. A secondary insult such as a precipitating illness was identified (for example: cold exposure, infection, drugs). We used these 6 criteria to develop an objective score to classify patients as definite, likely, or unlikely to have MC. We assigned points to each patient based on these 6 criteria: 1) GCS of 0-10 = 4, 11 - 13 = 3, 14 =2, 15 = 0; 2) TSH > 30 = 2, 15-30 = 1; 3) FT4 below normal = 1; 4) hypothermic (temp < 95 F) = 1; 5) bradycardic (HR < 60) =1; 6) precipitating illness present = 1. We then considered MC present if the score was >7, likely = 5 -7, unlikely < 5. Ten patients aged 68+/- 25 years old were admitted with a diagnosis of MC. All had a GCS of less than 15. Eight (80%) had a TSH of more than 30 [mu]U/ml. Six (60%) were found to be bradycardic. Five (50%) had an identifiable precipitating factor. Four (40%) were hypothermic. Based on our scoring system two (20%) patients had definite MC, 6 (60%) had likely MC and 2 (20%) were unlikely to have MC. The two patients unlikely to have MC had secondary hypothyroidism. We would recommend patients that have a score >7 be treated for MC; patients with scores from 5 to 7 also be treated if there are no other plausible causes for the findings. In summary, we developed an objective-screening tool to aid in the rapid diagnosis of MC. As the features of MC are non-specific, this screening tool enables physicians to rapidly make the diagnosis of MC to expedite immediate treatment. We propose this screening tool be used prospectively to create a more refined diagnostic tool for the rapid diagnosis of this rare but lethal syndrome and to study the optimal treatment of MC.

Nothing to Disclose: YVC, CNM

Pub # OR25-1

Session Information ORAL SESSION: TRANSLATIONAL - Neuroendocrine Tumors & Endocrine-Related Signaling in Solid Tumors (11:15 AM-12:45 PM)

Title A Randomized, Double-Blind, Placebo-Controlled, Multicenter Phase III Trial of Everolimus + Octreotide LAR vs. Placebo + Octreotide LAR in Patients with Advanced Neuroendocrine Tumors (NET) (RADIANT-2): Updated Safety Results

Author String D Gross, M Peeters, V Jehl, S Saletan, L Sideris
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Body **Background:** Patients with advanced NET have limited treatment options. In the phase III RADIANT-2 study of patients with advanced low- or intermediate-grade NET and a history of secretory symptoms associated with carcinoid syndrome, everolimus, an oral mTOR inhibitor, + octreotide LAR provided a clinically meaningful 5.1-month increase in median progression-free survival (PFS) compared to placebo + octreotide LAR (ESMO 2010 Abstract #LBA8). An updated safety analysis from this trial is presented. **Methods:** Patients were randomized to everolimus 10mg/d orally + octreotide LAR 30mg IM q28d (n=216), or placebo + octreotide LAR (n=213). Adverse events (AEs) were collected and coded to a preferred term using the Medical Dictionary for Regulatory Activities. AEs were graded using the National Cancer Institute's Common Terminology Criteria (v 3.0). The safety population included 426 patients (215 everolimus; 211 placebo). **Results:** Median safety follow-up was 31.1 months. Everolimus safety was consistent with the primary analysis (median follow-up 28 months). Most common drug-related AEs with everolimus + octreotide LAR vs placebo + octreotide LAR were stomatitis (47.4% vs 10.9%), rash (37.2% vs 12.3%), fatigue (31.6% vs 24.2%), and diarrhea (27.4% vs 15.6%). The majority of events were modest in severity (grade 1/2) and most were easily managed with dose modification/interruptions, concomitant medication, or dietary intervention. Fatigue (6.5% vs 2.8%), diarrhea (6.0% vs 2.4%), hyperglycemia (5.1% vs 0.5%), thrombocytopenia (4.7% vs 0%), and stomatitis (3.7% vs 0%) were the most frequent drug-related grade 3/4 events in the everolimus + octreotide LAR and placebo + octreotide LAR groups, respectively. Rate of drug-related AEs leading to discontinuation was 19.5% for everolimus + octreotide LAR vs 3.8% for placebo + octreotide LAR. Disease progression was the primary reason for treatment discontinuation in both arms. **Conclusions:** Safety of everolimus observed in this updated analysis is consistent with previous experience. The severity of the side effects was modest compared to that observed with traditional cytotoxic chemotherapy; events with everolimus were predominantly grade 1/2 in nature; grade 3/4 events were unusual. These findings provide further evidence of the favorable risk-benefit profile of everolimus in patients with advanced NET and a history of secretory symptoms associated with carcinoid syndrome.

Sources of Research Support: Novartis.

Disclosures: DG: Clinical Researcher, Novartis Pharmaceuticals; Novartis Pharmaceuticals; Medison; Consultant, Ipsen; Pfizer, Inc. VJ: Employee, Novartis Pharmaceuticals. SS: Employee, Novartis Pharmaceuticals. LS: Clinical Researcher, Novartis Pharmaceuticals; Astra Zeneca; Roche Pharmaceuticals; Sanofi-Aventis. Nothing to Disclose: MP

Pub # OR25-2

Session Information ORAL SESSION: TRANSLATIONAL - Neuroendocrine Tumors & Endocrine-Related Signaling in Solid Tumors (11:15 AM-12:45 PM)

Title A Randomized, Double-Blind, Placebo-Controlled, Multicenter Phase III Trial of Everolimus in Patients with Advanced Pancreatic Neuroendocrine Tumors (pNET) (RADIANT-3): Updated Safety Results

Author String D Horsch, C Lombard-Bohas, J Lincy, S Saletan, W Kocha
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Body
Background: In the phase III RADIANT-3 trial, everolimus, an oral mTOR inhibitor, demonstrated superiority in progression-free survival with a median of 11.0 vs 4.6 months for placebo (hazard ratio, 0.35; 95% confidence interval, 0.27 to 0.45; P<0.0001) in patients with advanced pNET (ESMO 2010, Abstract #LBA9). An update of the safety analysis from this trial is presented.
Methods: Patients with progressive advanced low- or intermediate-grade pNET were randomized to everolimus 10mg/d orally (n=207) or placebo (n=203); both arms received best supportive care. The primary endpoint was PFS. Adverse events (AEs) were collected and coded to a preferred term using the Medical Dictionary for Regulatory Activities. AEs were graded using the National Cancer Institute's Common Terminology Criteria (v 3.0). Safety population included 407 patients (204 for everolimus; 203 for placebo).
Results: Median study follow-up was 20.1 months. Safety of everolimus was consistent with the primary analysis (median follow-up 17 months). Median exposure to everolimus was 2.3-fold longer than placebo (37 vs 16 weeks), corresponding to exposures of 175.2 patient-years vs 104.9 patient-years, respectively. Most frequent drug-related AEs with everolimus vs placebo were mild to moderate in nature (i.e., grade 1/2) and included stomatitis (52.9% vs 12.3%), rash (48.5% vs 10.3%), diarrhea (34.3% vs 10.3%), and fatigue (32.4% vs 14.3%). Common drug-related grade 3/4 events for everolimus and placebo were anemia (5.9% vs 0%), hyperglycemia (5.9% vs 2.5%), stomatitis (4.9% vs 0), thrombocytopenia (3.9% vs 0%), and diarrhea (3.4% vs 0%), respectively. Most AEs resolved with dose modification/interruptions or concomitant medication. Rate of drug-related AEs leading to discontinuation was 13.7% in the everolimus group vs 2.0% in the placebo group. Disease progression remained the primary reason for discontinuation from both treatment arms.
Conclusions: Safety of everolimus in pNET patients observed in this updated analysis is consistent with previous experience. The severity of the side effects with everolimus was modest compared to that observed with traditional cytotoxic chemotherapy; events with everolimus were predominantly grade 1/2 in nature; grade 3/4 events were unusual. These findings provide further evidence of the favorable risk-benefit profile of everolimus in advanced pNET patients.

Sources of Research Support: Novartis.

Disclosures: DH: Clinical Researcher, Novartis Pharmaceuticals; Ipsen; Pfizer, Inc.; Eckart and Ziegler; Covidien and ITG. JL: Employee, Novartis Pharmaceuticals. SS: Employee, Novartis Pharmaceuticals. Nothing to Disclose: CL-B, WK

Pub # OR25-3

Session Information ORAL SESSION: TRANSLATIONAL - Neuroendocrine Tumors & Endocrine-Related Signaling in Solid Tumors (11:15 AM-12:45 PM)

Title The Role of Proinsulin in the Diagnosis of Insulinoma: A Critical Evaluation of the Endocrine Society Guidelines

Author String J-M Guettier, A Lungu, A Goodling, C Cochran, P Gorden
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Body Documenting Whipple's triad and concomitant elevation in plasma insulin or proinsulin in a fasted subject are key to the diagnosis of insulinoma. An end of fast plasma insulin value of 3 [micro]U/ml and plasma proinsulin value of 5 pmol/liter have been suggested as useful cut points for the diagnosis of insulinoma[i]. We evaluated the diagnostic accuracy of the proposed threshold values in subjects seen at NIH since our last published[ii] series and in the era of specific insulin immunoassays.

Study: Case-Control

Measured Outcomes: Diagnostic accuracy of plasma insulin (NIH Labs) and plasma proinsulin (Mayo Clinics Labs).

Result: 67 subjects with fasting hypoglycemia had a surgically confirmed insulinoma; 52 had non-recurrent disease limited to the pancreas, 8 had metastatic disease and 7 had underlying MEN-1. 43 were women and 24 were men. The mean age (\pm SD) was 46.1 (16.1) years. In 27 subjects a diagnosis of insulinoma was excluded 21 subjects had no identifiable hypoglycemic disorders at the time of evaluation and follow-up and 6 cases had factitious hypoglycemia due to sulfonylurea ingestion. 19 were women and 8 were men. The mean age (\pm SD) was 40.1 (13.6).

Three (95% sensitivity) insulinoma cases had measured end-of-fast insulin value of [le] 3 [micro]U/mL. Seven (89% sensitivity) had measured end-of-fast insulin value of [le] 5 [micro]U/mL (i.e., threshold value used for older insulin assays).

The median (Interquartile Range) end-of-fast proinsulin value was 100 (55-310) pmol/liter for insulinoma cases and 6.8 (4.3-14.0) pmol/liter for controls (Wilcoxon Rank test p-value < 0.0001). 100% of cases (100% sensitivity) and 64% of controls (36% specificity) had an end of fast proinsulin value of [ge] 5 pmol/liter. In our dataset sensitivity remained at 100% if the threshold was raised to an end-of-fast proinsulin value of [ge] 15.4 pmol/liter. At this cutpoint the false positive rate was 24% (76% specificity). The end-of-fast proinsulin value yielding the highest area under the ROC curve was 27 pmol/liter (Sensitivity 99% and False Positive Rate 4%).

All cases with an end-of-fast insulin value of [le] 3 or 5 [micro]U/mL had end-of-fast proinsulin values [ge] 42 pmol/liter.

Conclusion: Proinsulin remains a useful test in the era of newer insulin assays. The proposed end-of-fast proinsulin threshold of 5 pmol/liter value is too low to discriminate cases from controls. The value of proinsulin is particularly useful in cases where the end-of-fast insulin value suggests adequate suppression.

[i] Evaluation and Management of Adult Hypoglycemic Disorders: An Endocrine Society Guideline. J Clin Endocrinol Metab 94:709-728, 2009

[ii]Forty-Eight-Hour Fast. The Diagnostic Test for Insulinoma. J Clin Endocrinol Metab 94: 3222-3226, 2000

Sources of Research Support: Intramural Research Program, of National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health; U.S. Department of Health and Human Services.

Nothing to Disclose: J-MG, AL, AG, CC, PG

Pub # OR25-4

Session Information ORAL SESSION: TRANSLATIONAL - Neuroendocrine Tumors & Endocrine-Related Signaling in Solid Tumors (11:15 AM-12:45 PM)

Title Role of LKB1 in Adiponectin-Regulated Malignant Potential of Human Colon Cancer *In Vivo* and *In Vitro*

Author String H-S Moon, X Liu, KN Diakopoulos, JM Nagel, CS Mantzoros
Beth Israel Deaconess Medical Center, Boston, MA; Harvard School of Public Health, Boston, MA; Boston VA Healthcare System, Boston, MA

Body

Introduction: Low circulating adiponectin levels correlate with carcinogenesis of several malignancies including endometrial, breast, and prostate cancers. Adiponectin not only suppresses angiogenesis by inducing endothelial cell apoptosis but also inhibits tumorigenesis *in vivo*. Adiponectin, besides being a promising candidate for treating obesity-associated metabolic diseases, may be a potential candidate against novel therapies for tumor growth, recurrence, and metastasis. However, a detailed study of adiponectin in colon cancer has not yet been reported and adiponectin signaling in malignant colon cancer remains to be studied.

Methods: We utilized wild type (WT) and heterozygous adiponectin knockout (Het, adiponectin insufficient) mice which were randomized to either high fat (HF) or low fat (LF) diet, and investigated whether a low dose of adiponectin has a physiological protective role against the progression of carcinogenesis, angiogenesis, and inflammation *in vivo*. We then directly tested whether LKB1 is required in adiponectin-mediated modulation of the AMPK-S6 axis and inhibition of cell proliferation, colony formation, adhesion and invasion of human and mouse colon cancer cells.

Results: In animal *in vivo* studies, we found that adiponectin treatment inhibits cancer growth, reduces angiogenic protein expression, and causes extensive central necrosis of implanted colon cancer cells in WT and Het mice which were fed either a HF or LF diet. By contrast, natural killer (NK) cells have more infiltrated into tumors in Het mice compared to WT mice. In addition, NK cells were found in adiponectin treated Het mice on HF diet, but not LF diet. Improvement of HOMA-IR was only positively correlated with decreasing tumor weight in Het mice, but not WT mice. In human and mouse *in vitro* colon cancer cell line studies, we observed that adiponectin increases the activation of the adaptor molecules LKB1 which is required for adiponectin-mediated modulation of the AMPK-S6 axis and inhibition of malignant potential of colon cancer cells. Also, we observed that depletion of LKB1 by its siRNA administration abrogated adiponectin-mediated expression of tumor suppressor and cell cycle regulatory genes of human and mouse colon cancer cells.

Conclusions: These novel mechanistic studies provide evidence for a casual role of adiponectin in colon cancer and indicate that adiponectin could potentially prove to be a useful agent in the management of colon cancer.

Hardie DG. *Curr Opin Cell Biol* 2005; 17:167
Yamauchi et al., *Nature* 2003; 423:762
Kelesidis et al., *Br J Cancer* 2006; 94:1221

Sources of Research Support: National Institute of Diabetes and Digestive and Kidney Diseases grants DK58785, DK79929 and DK81913, and AG032030. The Mantzoros Laboratory is also supported by a discretionary grant from Beth Israel Deaconess Medical Center.

Nothing to Disclose: H-SM, XL, KND, JMN, CSM

Pub # OR25-5
Session Information ORAL SESSION: TRANSLATIONAL - Neuroendocrine Tumors & Endocrine-Related Signaling in Solid Tumors (11:15 AM-12:45 PM)
Title Activation of K-Ras May Play an Important Role in Endometrial Cancer by Inhibiting Progesterone Signaling
Author String MJ Large, TH Kim, JP Lydon, J-W Jeong, FJ DeMayo
Baylor College of Medicine, Houston, TX; Van Andel Research Institute, Michigan State University, Grand Rapids, MI

Body Endometrial cancer is the fourth most common cancer in women. Excessive estrogen (E2) action is associated with endometrial carcinoma and plays a very important role in the progression of the disease. E2 is stimulatory to epithelial proliferation and excessive E2 exposure results in endometrial hyperplasia. Progesterone (P4) ameliorates the proliferative effects of E2 and has been used in the treatment of endometrial cancer. Genetic alterations, such as mutations in the tumor suppressor Pten and the oncogene K-ras, are also associated with endometrial cancer progression. Recently, our lab generated mice with Pten ablation and oncogenic K-ras mutation in progesterone receptor positive cells (PR^{cre/+} Pten^{f/f} K-ras^{G12D}). Double mutant mice exhibited dramatically accelerated development of endometrial cancer compared to mice with a single mutation of either gene, displaying severe endometrial cancer with 100% penetrance by one month of age. To investigate the molecular mechanisms of endometrial cancer development in these mouse models, we conducted microarray analysis on the uteri of 2 week old mice. Compared to PR^{cre/+} controls, there were 3,296 genes altered in the PR^{cre/+} Kras^{G12D} mice, 8,228 genes altered in the PR^{cre/+} PTEN^{f/f} mice, and 10,911 genes altered in the bigenic mice. These results alone suggest that alteration of both genes results in an additive effect rather than a synergistic one. Examination of the effects of mutant K-ras alone indicates that there are alterations in the expression of molecules involved in estrogen receptor signaling, Wnt/[Beta]-catenin signaling, Hedgehog signaling, p53 signaling, and p38 MAPK signaling. Functional groupings of the altered genes demonstrates changes in 713 genes related to cancer, 582 genes related to cell growth and proliferation, 276 genes related to the cell cycle, and 361 genes known to be important in reproductive diseases. Interestingly, the PR^{cre/+} Kras^{G12D} uteri also exhibited decreased expression of many P4 target genes. Additionally, we show that K-ras activation inhibits the activation of PR target genes in response to P4. Thus, these data suggests that the activation of K-ras may play an important role in the etiology of endometrial carcinoma by repressing the effects of P4, resulting in unabated E2 actions.

Sources of Research Support: NIH Grant RO1CA077530 (to J.P.L.), by NIH R01 HD057873 (to JW. J), and by the Eunice Kennedy Shriver NICHD/NIH through cooperative agreement U54 HD07495 as part of the Specialized Cooperative Centers Program in Reproduction and Infertility Research.

Nothing to Disclose: MJL, THK, JPL, J-WJ, FJD

Pub # OR25-6

Session Information ORAL SESSION: TRANSLATIONAL - Neuroendocrine Tumors & Endocrine-Related Signaling in Solid Tumors (11:15 AM-12:45 PM)

Title Prostate Apoptosis Response-4 (Par-4): A Novel Substrate of Caspase-3 during Apoptosis Activation in Hormone-Dependent Tissues

Author String E Asselin, P Chaudhry, M Singh, S Parent
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Body Prostate apoptosis response-4 (Par-4) is a ubiquitously expressed pro-apoptotic tumor suppressor protein. Here, we show for the first time, that Par-4 is a novel substrate of caspase-3 during apoptosis. In the present study we used human ovarian cancer cell lines (A2780-S, A2780-CP, OVCAR-3), human endometrial carcinoma cell lines including (KLE, Ishikawa, Hec-1-A), human cervical carcinoma cell line (Hela), human prostate carcinoma cell line (PC3), human breast cancer cell lines (MCF-7 and MDA-468), human normal uterine epithelial (CL22) and normal uterine stromal (Hend2) cells. We found that Par-4 is cleaved during cisplatin-induced apoptosis in human normal and cancer cell lines. Caspase-3-deficient MCF-7 cells did not show Par-4 cleavage in response to cisplatin treatment. This Par-4 cleavage was concomitant with Parp cleavage and generated a C-terminal fragment of ~25kDa. The cleavage of Par-4 was completely inhibited by Z-VAD-fmk (an inhibitor of caspase like protease) suggesting that caspase-3 like activity is involved in the cleavage of Par-4. Consistently, recombinant caspase-3 efficiently cleaved Par-4 *in vitro for all cell lines tested*, suggesting the involvement of this protease in Par-4 processing *in vivo*. Overexpression of caspase-3 in PC-3 cells increased caspase-3 activity, which further resulted in the cleavage of Par-4 in these cells. However, overexpression of a catalytic mutant caspase-3 (C163A) failed to induce Par-4 cleavage. Further, restoration of caspase-3 in MCF-7 cells showed a decrease in Par-4 levels with the appearance of the cleaved fragment. The cleavage of Par-4 in caspase-3 expressing MCF-7 cells was efficiently blocked by a caspase-3 inhibitor. Interestingly, Par-4 levels were also found to be regulated by caspase-3 under normal physiological conditions during the rat estrous cycle. These results establish for the first time that Par-4 is a novel substrate of caspase-3 both *in vitro* and *in vivo*. Additionally, we found that Par-4 expression was down regulated in advanced grade ovarian and endometrial carcinomas indicating a possible role during cancer progression.

Nothing to Disclose: EA, PC, MS, SP

Pub # OR26-1

Session Information ORAL SESSION: CLINICAL - Cardiometabolic Risk: Inflammation & Insulin Resistance (11:15 AM-12:45 PM)

Title Sitagliptin Exerts an Anti-Inflammatory Effect

Author String P Dandona, A Makdissi, CL Sia, M Vora, K Korzeniewski, H Ghanim
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Body Sitagliptin is an inhibitor of the enzyme dipeptidyl peptidase-IV (DPP-IV), which degrades the incretins, GLP-1 and GIP, and thus increases their bio-availability. The stimulation of insulin and the suppression of glucagon secretion that follow exert a glucose lowering effect and hence its use as an anti-diabetic drug. Since DPP-IV is expressed as CD26 on cell membranes and since CD26 mediates pro-inflammatory signals, we hypothesized that sitagliptin may exert an anti-inflammatory effect. Twenty two patients with type 2 diabetes were randomized to receive either 100 mg daily of sitagliptin (n=12, mean age: 54.4±4 years; mean BMI: 34.8±1.3kg/m²; mean HbA1c:7.6±0.1%) or placebo (n=10, mean age: 55.7±4.5 years; mean BMI: 35.1±1.5kg/m²; mean HbA1c:7.9±0.3%) for 12 weeks. Fasting blood samples were obtained at 0, 2, 4, 8 and 12 weeks. There was no significant change in fasting blood glucose concentrations while HbA1c fell significantly from 7.6±0.1% to 6.9±0.3% in patients treated with sitagliptin. Fasting GLP-1 concentrations increased significantly by 63±20% (from 9.1±2.8 to 15.8±4.0pM) following sitagliptin treatment. In addition, the mRNA expression in MNC of the pro-inflammatory cytokine, TNF α , the receptor for endotoxin, TLR-4, and pro-inflammatory kinases, JNK-1 and IKK β fell by 39±10%, 23±11%, 19±8% and 17±9%, respectively, while that of the chemokine receptor CCR-2 fell by 24±8% (P<0.05, all). This was accompanied by a significant fall of plasma concentrations of CRP, IL-6 and free fatty acids by 24±7%, 24±8% and 19±11%, respectively (P<0.05, all). In addition, the expression of CD26 in MNC was suppressed by 23±7% following sitagliptin treatment. While these effects are consistent with a potent anti-inflammatory effect of sitagliptin and may potentially contribute to the inhibition of atherosclerosis, the suppression of CD26 expression is surprising since we recognize sitagliptin as an inhibitor of DPP-IV action but not its production. We conclude that sitagliptin exerts a potentially beneficial anti-inflammatory effect. Its relationship to CD26 and DPP-IV requires further investigation.

Sources of Research Support: NIH Grants R01-DK075877 and R01-DK069805 awarded to PD; American diabetes Grant awarded to PD.

Disclosures: PD: Principal Investigator, GlaxoSmithKline; Clinical Researcher, Sanofi-Aventis; Speaker, Novartis Pharmaceuticals; Eli Lilly & Company; GlaxoSmithKline; Sanofi-Aventis. Nothing to Disclose: AM, CLS, MV, KK, HG

Pub # OR26-2
Session Information ORAL SESSION: CLINICAL - Cardiometabolic Risk: Inflammation & Insulin Resistance (11:15 AM-12:45 PM)

Title Effects of Ergocalciferol on Insulin Resistance in Healthy Men and Women with Low Vitamin D Levels

Author String S-AM Burnett-Bowie, E Cagliero, N Mendoza, DL Hayden, JS Finkelstein, BZ Leder
Massachusetts General Hospital, Boston, MA

Body Epidemiologic data suggest that low 25-hydroxyvitamin D (25OHD) levels increase insulin resistance (IR) and the risk for type 2 diabetes mellitus (T2DM). There are, however, few interventional trials assessing the effect of vitamin D on IR and the published results are discordant. Furthermore, many of these interventional studies have been performed in Caucasian cohorts who are at decreased risk for vitamin D deficiency and T2DM. **Methods:** We randomized 90 healthy men and women, aged 18-45, with 25OHD levels ≤ 20 ng/mL by chemiluminescent immunoassay (*DiaSorin*, Stillwater, MN) to weekly ergocalciferol (VIT-D) 50,000 international units or placebo (PBO) for 12 weeks. Both groups consumed 1000-1500 mg calcium daily. The study was double-blinded and randomization was stratified by normal vs. impaired glucose tolerance (IGT) or screening 2 hour oral glucose tolerance test (OGTT), sex, and 25OHD ≤ 10 vs. > 10 ng/mL. The primary endpoint was the difference in the between group change in IR by modified intravenous glucose tolerance test (mIVGTT). A mIVGTT assesses both IR and insulin secretion (IS) during the same dynamic test. Secondary endpoints included the difference in the between group change in IS by mIVGTT, homeostasis model assessment (HOMA)-IR, glucose, insulin, cholesterol, weight, and blood pressure. The mIVGTT and cholesterol endpoints were assessed at weeks 0 and 12, the other endpoints were assessed at weeks 0, 4, 8, and 12. **Results:** Subjects were racially diverse (52/90 were non-Caucasian); 9/90 had IGT; and mean HOMA-IR values were 2.0 ± 1.1 and 2.2 ± 1.7 in the VIT-D and PBO groups respectively. In the VIT-D group, mean 25OHD (by liquid chromatography/tandem mass spectroscopy) increased from 18 ± 7 ng/mL to 40 ± 12 ng/mL at week 4 ($p < 0.0001$) and remained stable at 43 ± 12 ng/mL at week 12 ($p < 0.0001$); mean $1,25(\text{OH})_2\text{D}$ increased from 42 ± 17 pg/mL to 52 ± 18 pg/mL at week 4 ($p < 0.001$) and then remained stable. Changes in IR (by mIVGTT or HOMA) and IS were similar in the VIT-D and PBO groups. There were no between group differences in the other endpoints. The treatment effect was not affected by IGT, sex, or 25OHD level. **Conclusions:** In healthy persons with low 25OHD and mostly normal HOMA-IR, ergocalciferol administration for 12 weeks normalizes 25OHD levels but does not improve insulin sensitivity or secretion. Future studies on the effect of VIT-D on IR should be of longer duration and/or include greater numbers of subjects with IGT.

Sources of Research Support: NIH grants K23-DK-073356 to SMB and M01-RR-01066; the Massachusetts General Hospital Physician-Scientist Development Award; and the Boston Area Diabetes and Endocrinology Research Center Grant.

Nothing to Disclose: S-AMB-B, EC, NM, DLH, JSF, BZL

Pub # OR26-3

Session Information ORAL SESSION: CLINICAL - Cardiometabolic Risk: Inflammation & Insulin Resistance (11:15 AM-12:45 PM)

Title Novel Compound Heterozygous Mutations of Insulin Receptor Found in Leprechaunism: Case Report

Author String R Nishimura, M Fujimoto, N Miyahara, Y Kawashima, K Hanaki, S Kanzaki
Faculty of Medicine Tottori University, Yonago, Japan; Faculty of Medicine Tottori University, Yonago, Japan

Body Leprechaunism is an extremely rare disorder characterized by resistance to insulin due to abnormal insulin receptor (IR) and distinctive clinical characteristics; low birth weight, characteristic facial features and acanthosis nigricans. Recently we experienced a patient with leprechaunism bearing novel compound heterozygous mutations of IR (Thr910Met and Glu1047Lys).
Eight-day-old Japanese boy was referred to our hospital because of poor sucking and poor activity. He was delivered at 41wk gestation with birth weight 1920g (-3.7SD). He had characteristic appearance with acanthosis nigricans, lack of subcutaneous fat, decreased muscle, hirsutism, prominent eyes, low-set large ears, nasal bridge, thick lips and gum hypertrophy. Plasma glucose levels ranged from 38 mg/d L in the fasting state to over 200 mg/dL postprandially. Fasting plasma insulin level was greatly increased at 6702 mU/mL. As he was suggested to have IR anomaly, and his weight gain was extremely poor, recombinant human IGF-I (rhIGF-I) treatment was started at day 24. However, rhIGF-I treatment did not improve poor weight gain, though his serum insulin level was improved. At 1-month-old, he was noticed both hyperbilirubinemia and liver dysfunction, and was diagnosed as cholestasis. He is now 1-year -old, although cholestasis is improved, his poor weight gain has not been improved despite rhIGF-I treatment. IR gene analysis using mononuclear cells revealed he had compound heterozygous mutation (Thr910Met/Glu1047Lys). The detected mutation (Thr910Met) is previously reported, and associated with impaired processing of pro-insulin receptor. However, Glu1047Lys mutation has not been reported. Glu1047Lys mutation sited at the kinase domain of the IR β subunit and is thought to be involved in interaction with ATP. Functional analysis of this novel IR gene mutation should be warranted. RhIGF-I treatment has been used for patients with IR gene mutation. However, rhIGF-I could not improved the symptoms of our patient. Novel Glu1047Lys missense mutation might add new informations as for IR function.

Nothing to Disclose: RN, MF, NM, YK, KH, SK

Pub # OR26-4

Session Information ORAL SESSION: CLINICAL - Cardiometabolic Risk: Inflammation & Insulin Resistance (11:15 AM-12:45 PM)

Title Sleep Disturbances in Pregnancy and Their Relationship to Glucose Tolerance (GT) and Gestational Diabetes (GDM)

Author String S Reutrakul, W Kristen, N Zaidi, M Ismail, HH Kay, R Schneider, DA Ehrmann, E Van Cauter
Rush University Medical Center, Chicago, IL; University of Chicago, Chicago, IL; University of Chicago, Chicago, IL; University of Chicago, Chicago, IL

Body Sleep disordered breathing (SDB) is associated with insulin resistance and diabetes. The extent to which SDB occurs and impacts GT in pregnancy has not been well characterized. We explored sleep quality and SDB, in relationship to GT and pregnancy outcomes in 169 women undergoing a 50gm OGTT, typically at 24-28 weeks of gestation. The Pittsburg Sleep Quality Index (PSQI), the Epworth Sleepiness Scale (ESS) and the Berlin questionnaire to evaluate SDB risk were administered. On average, sleep quality was poor with a mean \pm SD PSQI of 7.4 ± 4.0 (normal ≤ 5). Mean ESS was 8.0 ± 4.6 (normal ≤ 8), reflecting daytime sleepiness. 29% were at increased risk for SDB, 92% had interrupted sleep and 52% reported sleeping < 7 hr/night (short sleep [SS]).

116 women (69%) had a normal 50g OGTT (NGT), and 26 (15%) had GDM. Sleep disturbances predicted an increased likelihood of GDM with an odds ratio of 3.0 (95% CI 1.2-7.4) for high SDB risk ($p < 0.05$), 2.4 (95% CI 1.0-5.9) for SS ($p = 0.06$), 3.4 (95% CI 1.3-8.7) for the combination of high SDB risk and SS (SDB+SS) ($p = 0.01$), and 3.4 (95% CI 1.3-8.8) for snoring $> 3-4$ night/week ($p = 0.01$, BMI adjusted)

Fifteen GDM women had an overnight polysomnography, 11 (73%) of whom had an apnea-hypopnea index ≥ 5 , the cutoff for SDB. Ten NGT women matched for age, BMI and ethnicity served as controls. Compared to the NGT group, GDM women had lower total sleep time (393 ± 75 vs. 447 ± 44 min, $p < 0.05$), more fragmented sleep as reflected by a higher microarousal index (24.7 ± 10.3 vs. 17.2 ± 5.3 , $p = 0.05$) and an increased incidence of SDB (90% vs. 40%, $p = 0.06$).

Pregnancy outcomes were analyzed in 134 women (108 NGT, 26 GDM). The GDM group had a higher prevalence of gestational hypertension (23% vs. 5%, $p < 0.01$); as expected, fetal birth weight was higher (3666 ± 597 vs. 3232 ± 413 gm, $p < 0.01$), and NICU admission more frequent (46% vs. 18%, $p < 0.01$). After adjustment for GT status, preterm delivery was more frequent in those with an increased PSQI ($p = 0.06$), SS ($p = 0.05$) and SDB+SS ($p = 0.06$). NICU admissions were associated with higher ESS ($p = 0.05$) and SDB+SS ($p = 0.03$). Primary C-section was more frequent in subjects who reported snoring ($p = 0.02$).

Summary: Pregnant women experience significant sleep disturbances, which are associated with increased risk of GDM and unfavorable pregnancy outcomes. GDM women have a higher prevalence of SDB and poor sleep quality, independent of BMI. These findings suggest that sleep disturbances contribute to the risk of diabetes in pregnancy.

Sources of Research Support: The ResMed Foundation. The sponsor had no role in the study design, data collection, analysis, interpretation or writing of the report; the study is also supported by Grant Number UL1RR024999 from the National Center For Research Resources. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Center For Research Resources or the National Institutes of Health.

Nothing to Disclose: SR, WK, NZ, MI, HHK, RS, DAE, EVC

Pub # OR26-5

Session Information ORAL SESSION: CLINICAL - Cardiometabolic Risk: Inflammation & Insulin Resistance (11:15 AM-12:45 PM)

Title Gestational Diabetes Mellitus Is Associated with Cardiovascular Disease Differentially by Race

Author String R Bentley-Lewis, CE Powe, E Ankers, JB Wenger, JL Ecker, RI Thadhani
Massachusetts General Hospital, Boston, MA; Massachusetts General Hospital, Boston, MA; Massachusetts General Hospital, Boston, MA

Body

Background: Gestational diabetes mellitus (GDM) is a risk factor for maternal cardiovascular disease (CVD) following pregnancy. However, identification of CVD events in women with a history of GDM has been limited by non-clinical data and variable follow-up of this population. Moreover, the impact of race has not been fully explored.

Objectives: To test the hypotheses that women with GDM demonstrate a greater risk for subsequent CVD compared to women without GDM and that this relationship will be modified by race.

Participants/Interventions: We conducted a case-control study of women who delivered at Massachusetts General Hospital between 1998-2007. GDM was defined by Carpenter-Coustan criteria and ICD-9 codes were used to identify diabetes mellitus, CVD, cerebrovascular disease, ischemic heart disease, and essential hypertension (HTN). Women with these outcomes prior to delivery were excluded. GDM cases were matched by gravidity to controls (1:4) without GDM. Cox Proportional Hazards regression was performed for GDM, demographic and clinical characteristics, and for each cardiovascular disease outcome. Data were censored if diabetes developed prior to a cardiovascular outcome. If no outcome developed, women were censored based on their last admission.

Results: There were 802 women with GDM and 3,208 women without GDM followed for a median of 3.6 years (maximum 11.9 years). GDM was significantly associated with older age; elevated pregnancy body mass index and systolic (SBP) and diastolic blood pressures; and frequency of non-white race, CVD and HTN compared to the non-GDM group. GDM predicted future CVD (HR 1.32, 95% CI 1.11-1.56) and HTN (HR 2.68, 95% CI 2.07-3.48) in univariate analysis, but only HTN (HR 2.30, 95% CI 1.71-3.08) remained significant after adjusting for age, SBP, parity, and maternal pregnancy weight gain. Black (HR 1.56, CI 95% 1.14-2.13) and Hispanic (HR 1.40, CI 95% 1.13-1.74) race predicted CVD even after these adjustments. Notably, in stratified analysis by race, Hispanic women with GDM were more likely to develop CVD than Hispanics without GDM (HR 1.70, CI 95% 1.60-2.41).

Conclusion: GDM predicts future CVD, but not independently of risk factors such as age and SBP. Hispanic women with GDM develop CVD to a greater degree than would be predicted by either GDM or Hispanic race alone. These results support the need for enhanced postpartum CVD risk surveillance and prevention efforts, even among young women, with a prior history of GDM.

Sources of Research Support: NIH 1K23RR023333 and the Robert Wood Johnson Foundation Harold Amos Medical Faculty Development Program awarded to R.B-L. Howard Hughes Medical Institute Medical Research Fellowship, 2009-2010, awarded to C.E.P.

Nothing to Disclose: RB-L, CEP, EA, JBW, JLE, RIT

Pub # OR26-6

Session Information ORAL SESSION: CLINICAL - Cardiometabolic Risk: Inflammation & Insulin Resistance (11:15 AM-12:45 PM)

Title Gestational Diabetes Mellitus Is Associated with Breast Cancer within 10 Years of Pregnancy

Author String CE Powe, R Bentley-Lewis, E Ankers, JB Wenger, J Ecker, R Thadhani
Massachusetts General Hospital and Harvard Medical School, Boston, MA; Massachusetts General Hospital and Harvard Medical School, Boston, MA; Massachusetts General Hospital and Harvard Medical School, Boston, MA

Body

Background: Pregnancy confers a short-term increase in the risk of breast cancer, and breast cancer that occurs in close proximity to pregnancy is associated with increased mortality. Type 2 diabetes mellitus is also associated with breast cancer. However, it is not known if gestational diabetes mellitus (GDM) is associated with an increased risk of future breast cancer.

Objectives: To test the hypothesis that GDM identifies a population of women at increased risk for developing breast cancer.

Participants/Interventions: We conducted secondary analysis of a cohort of women who delivered infants at Massachusetts General Hospital (MGH) between 1998 and 2007. We included pregnancies (the first in the study period for a given woman) screened for GDM (n=17, 241). Carpenter-Coustan criteria were used to classify GDM. We obtained ICD-9 codes for inpatient and outpatient discharge diagnoses occurring between the first prenatal visit and December 2009. Logistic regression was used to test for an association between GDM and cancer (any neoplasm, ICD-9 codes 140-239), including breast cancer (ICD-9 code 174). Length of follow-up was ascertained at the last visit to MGH.

Results: GDM occurred in 4.1% of the study population. Median length of follow up was 4.1 years (max 11.1 years). GDM was associated with older age, higher first trimester blood pressures, larger body mass index, longer length of follow-up, non-white race, and multiparity. Breast cancer was associated with older age, higher first trimester blood pressures, white race, and longer length of follow-up. Breast cancer occurred in 142 (0.78%) women at a median of 4.0 years after delivery. GDM was associated with an increased odds of all neoplasms (OR 1.21, 95% CI 1.01-1.44) and notably breast cancer (OR 2.05, 95% 1.10-3.81). GDM remained associated with breast cancer (OR 2.01, 95% CI 1.05-3.83) after adjusting for length of follow-up, age, race, and obesity, but there was no association between GDM and all neoplasms after adjustment for these factors (p=0.704). The development of type 2 diabetes mellitus subsequent to delivery did not explain the association between GDM and breast cancer.

Conclusion: GDM is associated with breast cancer occurring within 10 years of pregnancy. These data identify a group of women who may benefit from enhanced surveillance, although further investigation is needed to confirm these findings and account for possible confounding.

Sources of Research Support: C.E.P. is a 2009-2010 Howard Hughes Medical Institute Medical Research Fellow. This work was supported in part by grants from the NIH 1K23RR023333 and the Robert Wood Johnson Foundation Harold Amos Medical Faculty Development Program awarded to R.B.-L.

Nothing to Disclose: CEP, RB-L, EA, JBW, JE, RT

Pub # OR27-1

Session Information ORAL SESSION: CLINICAL - Growth Hormone & IGFs: Links to Cancer, Metabolism & Mortality (11:15 AM-12:45 PM)

Title Assessment of Primary Cancers in Growth Hormone-Treated Adult Hypopituitary Patients Compared to Population Databases: An Analysis of the Hypopituitary Control and Complications Study (HypoCCS)

Author String CJ Child, AG Zimmermann, WW Woodmansee, DM Green, JJ Li, H Jung, EM Erfurth, LL Robison Eli Lilly and Company, Windlesham, UK; Eli Lilly and Company, Indianapolis, IN; Brigham and Women's Hospital/Harvard Medical School, Boston, MA; St Jude Children's Research Hospital, Memphis, TN; Eli Lilly and Company, Bad Homburg, Germany; Sk[aring]nes University Hospital, Lund, Sweden

Body GH treatment has been suggested to influence the occurrence and/or progression of neoplastic disease following the identification of 2 cases of colorectal cancer in adults previously treated with human pituitary GH during childhood (1), and the report of increased risk for second neoplasms in GH-treated childhood cancer survivors (2). However, the impact of long-term GH therapy on the incidence of cancer in adult patients (pts) with GH deficiency (GHD) remains unknown. We assessed the reported primary cancer occurrence in the multinational HypoCCS observational study and compared the observed cases to expected cases using SEER for the USA (3) and GLOBOCAN (4) for all other countries. To our knowledge, this is the first reported prospective large-scale evaluation of primary cancer risk in adult pts with GHD receiving GH therapy during adulthood. Study data and serious adverse event reports from pts enrolled in HypoCCS with [ge]1 follow-up visit available (GH-treated pts, N=6840; non-treated pts, N=940 [mainly from the USA]) were examined for potential primary cancer cases. The mean (\pm SD) follow-up per patient was 3.7 (\pm 2.9) y for GH-treated and 2.5 (\pm 2.4) y for non-treated pts. After initial assessment to eliminate obvious non-cancer events and recurrences, potential cancer cases were reviewed by 3 independent reviewers to identify cases for analysis. The standardized incidence ratio (SIR) and 95% confidence interval (CI) for primary cancers in GH-treated pts was 0.88 (0.74-1.04) for all countries. SIRs for the US cohort were 0.94 (0.73-1.18) for GH-treated pts and 1.16 (0.76-1.69) for non-treated pts. The most frequent cancer types observed in GH-treated pts were prostate (N=24), breast (N=16), malignant melanoma (N=15), lung (N=11) and colorectal (N=11). Due to the previous concern regarding the latter (1), colorectal cancers were analyzed specifically; 11 cases were identified in GH treated pts from all countries, with an overall SIR of 0.54 (0.27-0.98) and for US pts only, 6 cases with a SIR of 0.84 (0.31-1.83). In summary, we did not find an increased risk for all-sites or colorectal primary cancers in the HypoCCS cohort when compared to general population cancer registries and a similar SIR was found in the GH-treated and non-treated groups in the USA. Nevertheless, as these findings would be strengthened by longer follow-up time, ongoing surveillance of GH-treated adult pts, especially those with history of previous neoplastic disease, remains critical.

- (1) Swerdlow AJ et al., *Lancet* 360:273
- (2) Ergun-Longmire B et al., *JCEM* 91:3494
- (3) Ries LAG et al. (http://seer.cancer.gov/csr/1975_2004/)
- (4) Globocan 2002. IARC. (<http://www-dep.iarc.fr/>)

Sources of Research Support: Eli Lilly and Company.

Disclosures: CJC: Employee, Eli Lilly & Company. AGZ: Employee, Eli Lilly & Company. WWW: Consultant, Previous employee, Scientific Board Member, Eli Lilly & Company. JLL: Employee, Eli Lilly & Company. HJ: Employee, Eli Lilly & Company. EME: Scientific Board Member, Speaker, Eli Lilly & Company. LLR: Scientific Board Member, Speaker, Eli Lilly & Company. Nothing to Disclose: DMG

Pub # OR27-2

Session Information ORAL SESSION: CLINICAL - Growth Hormone & IGFs: Links to Cancer, Metabolism & Mortality (11:15 AM-12:45 PM)

Title Does Growth Hormone Replacement Therapy Reduce Mortality in Adults with Growth Hormone Deficiency? -- Data from the Dutch National Registry of Growth Hormone Treatment in Adults

Author String CC van Bunderen, C van Nieuwpoort, LI Arwert, MW Heymans, AAM Franken, HPF Koppeschaar, AJ van der Lely, ML Drent
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Body

Introduction
Adults with growth hormone deficiency (GHD) have a decreased life expectancy due to cardiovascular diseases (CVD). Recombinant growth hormone (GH) treatment has made replacement therapy an option in adults with GHD. Short-term studies have shown beneficial effects on the metabolic profile. Data on long-term efficacy and safety are limited and the implication for life expectancy remains to be established.

Methods
The Dutch National Registry of Growth Hormone Treatment in Adults was founded in 1998 to gain more insight into long-term efficacy and safety of GH therapy in GHD adults in the Netherlands. A treatment group (n=2229), primary control group (n=109), and secondary control group (n=356) were retrospectively monitored with a median follow up of 6.1 year (interquartile range (IQR) 6.5). Standardized mortality ratios (SMR) for all-cause and cause-specific mortality were calculated. Expected mortality was obtained from cause, sex, calendar year and 5-year age-specific death rates obtained from death and population counts from the Dutch Central Bureau of Statistics.

Results
Of the 2694 patients 135 had died at a median age of 62.1 year (IQR 21.6). The SMR for all-cause mortality for men was 1.06 (95% confidence interval (CI) 0.81-1.40) and for women 1.66 (CI 1.23-2.23) in the treatment group. The risk on CVD mortality was increased in women (SMR 2.52; CI 1.57-4.06) and not in men. There was no increased risk on cancer mortality in the treatment group. The SMR in the control groups were not significantly elevated. Excluding high-risk patients (craniopharyngioma and other possible malignant causes of hypopituitarism) lead to normalization of the SMR for all-cause mortality. The risk on CVD mortality in women remained elevated.

Conclusion
GH deficient women receiving GH treatment have a higher SMR for all-cause mortality than men, which normalized after exclusion of high-risk patients. The risk on CVD mortality remained increased in women. There is no increased risk on cancer mortality in GH treated adults compared to the background population.

Nothing to Disclose: CCvB, CvN, LIA, MWH, AAMF, HPFK, AJvdL, MLD

Pub # OR27-3

Session Information ORAL SESSION: CLINICAL - Growth Hormone & IGFs: Links to Cancer, Metabolism & Mortality (11:15 AM-12:45 PM)

Title Insulin Sensitivity and Beta Cell Function in Isolated Growth Hormone Deficiency

Author String CRP Oliveira, R Salvatori, JAS Barreto-Filho, IES Rocha, RMC Pereira, A Mari, VC Campos, M Menezes, ES Gomes, RA Meneguz-Moreno, VP Araujo, NTF Leite, AC Nascimento-Junior, MIT Farias, TAR Vicente, RDC Araujo, EV Melo, MH Aguiar-Oliveira
Federal University of Sergipe, Aracaju, Brazil; Johns Hopkins University, Baltimore, MD; National Research Council, Padova, Italy

Body
Introduction: Complex relationships exist between growth hormone (GH) and glucose metabolism. GH is known to reduce insulin sensitivity, while its effector (IGF-1) increases it. Also, GH and IGF-1 have been involved in the regulation of growth and development of the islet cells. The aim of this study was to assess insulin sensitivity (IS) and beta cell function in patients with isolated GH deficiency (IGHD) due to a homozygous mutation in the GH releasing hormone receptor gene.
Patients and Methods: Oral glucose tolerance tests (1.75 g/Kg in IGHD and 75 g in Controls (C)) were performed with glucose and insulin measurement at 0[acute],30[acute],60[acute],90[acute],120[acute]and 180 [acute]in 24 adult GH-naive IGHD subjects (12 M; 39.21 ±1.7 yrs) and 25 C subjects (14 M; 39.9±12.5 yrs). Despite a trend of reduction in BMI (22.9±4.7 in IGHD and 25.3±3.9 in C, p=0.054), waist/hip (W/H) ratio was higher in IGHD (p=0.046). IS was assessed by HOMA_{air}; QUICKI, oral glucose insulin sensitivity in 2 hours (OGIS₂) and OGIS₃ (3 hours). Beta cell function was assayed by HOMA-beta, insulinogenic index, and area under the curve of insulin/glucose ratio (AUC I/G).
RESULTS: Glucose levels were higher during OGTT (p<0.0001), while insulin response presented a trend toward reduction (p=0.08) in IGHD. The number of patients with glucose intolerance was higher (p= 0.001) in the IGHD group (9 vs. 3). As previously reported, HOMA_{air} was lower (p= 0.04) in IGHD subjects, suggesting increased sensitivity. Similarly, QUICKI and OGIS₂ showed a non-significant trend toward elevation (p= 0.066 and p= 0.09, respectively), again implying enhanced sensitivity in IGHD. OGIS₃ showed no difference between the two groups. Beta cell function was reduced in the IGHD group, with reduction in basal insulin secretion (HOMA-beta, p=0.015), reduced rapid phase of insulin secretion (insulinogenic index, p<0.0001), and reduced global insulin secretion (AUC I/G, p=0.02).
CONCLUSIONS: Increased IS may reduce atherosclerosis despite the negative cardiovascular risk profile (increased total and LDL cholesterol, C-reactive protein and blood pressure), and may explain the normal longevity of this cohort. Reduced beta cell function in the IGHD group suggests the importance of a normal GH/IGF-1 axis for insulinogenic function, and may explain the findings of elevated glucose levels during OGTT and higher prevalence of impaired glucose tolerance in this group. The enhanced insulin sensitivity may protect them from developing frank type 2 diabetes.

Nothing to Disclose: CRPO, RS, JAB-F, IESR, RMCP, AM, VCC, MM, ESG, RAM-M, VPA, NTFL, CAN-J, MITF, TARV, RDCA, EVM, MHA-O

Pub # OR27-4

Session Information ORAL SESSION: CLINICAL - Growth Hormone & IGFs: Links to Cancer, Metabolism & Mortality (11:15 AM-12:45 PM)

Title Long-Term Growth Hormone (GH) Administration Increases Hepatic Insulin Resistance, but Not Muscle Insulin Resistance, in Healthy Older Men and Women

Author String H-W Lee, R Muniyappa, SM Harman, MR Blackman
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Body
Background: Normal aging is associated with reduced GH and IGF-I production, increased total body and visceral adiposity, decreased lean body mass, and worsened insulin resistance. Several studies suggest that acute and chronic GH replacement in younger patients with adult GH deficiency worsen hepatic insulin resistance. However, the effects of chronic GH supplementation on hepatic and muscle insulin resistance in healthy older individuals are unknown.
Objective: We examined baseline relationships among serum IGF-I concentrations, measures of adiposity, age, and hepatic and muscle insulin resistance (HIR and MIR, respectively) in 101 older (age 65-88 yr) men (n = 61) and women (n = 40) with age-related declines in IGF-I ([Ie] 230 ng/ml). We then assessed the effects of GH administration (20 [micro]g/kg, given subcutaneously 3x/week) for 26 weeks on surrogate indices of HIR and MIR derived from oral glucose tolerance testing (OGTT) in a subset of 46 of these subjects (age range: 65-83 yrs), using a double-masked, placebo-controlled randomized study design (Men: GH group, n = 14 vs. Placebo group, n = 12; Women: GH group, n = 10 vs. Placebo group, n = 10).
Methods: Measures of body composition were assessed by body mass index (BMI), DEXA [absolute and % total body fat (TBF), lean body mass (LBM)], and abdominal MRI [total (TAF), subcutaneous (SC fat) and visceral fat (VF)]. Surrogate indices of HIR and MIR were derived from circulating insulin and glucose concentrations during a 75g standard OGTT.
Results: Linear regression analysis of baseline measures revealed that IGF-I concentrations were directly related to VF (r = 0.32, P = 0.01) and HIR (r = 0.33, P = 0.02) in men. In contrast, IGF-I was not significantly related to any measures of adiposity, HIR, MIR or age in women. GH supplementation for 26 weeks increased LBM and decreased TBF (P < 0.05) in men and women, but did not affect VF (P > 0.05) in either sex. Despite these overall body composition changes, GH supplementation increased HIR, compared to placebo, in men and women (change from baseline: Men, 56.4% vs. -4.8%, P = 0.02; Women, 142% vs. 17.4%, P = 0.01, adjusted for baseline HIR values and changes in VF from baseline), but did not significantly affect MIR in either sex.
Conclusions: In healthy older men and women, as in younger patients with adult GH deficiency, long-term GH administration increases hepatic insulin resistance with little or no effect on skeletal muscle insulin resistance.

Sources of Research Support: In part by the Intramural Research Programs of the National Center for Complementary and Alternative Medicine, Bethesda, Maryland and the National Institute on Aging, Baltimore, Maryland; National Institutes of Health Research Grant RO-1 AG11005; General Clinical Research Center Grant MO-1-RR-02719, from the National Center for Research Resources, National Institutes of Health, Bethesda, MD 20892; and the Research Service, Veterans Affairs Medical Center, Washington DC.

Nothing to Disclose: H-WL, RM, SMH, MRB

Pub # OR27-5

Session Information ORAL SESSION: CLINICAL - Growth Hormone & IGFs: Links to Cancer, Metabolism & Mortality (11:15 AM-12:45 PM)

Title Effects of GH on Body Composition and Cardiovascular Risk Markers in Women with Visceral Adiposity: A 6-Month Randomized, Double-Blind Placebo Controlled Trial

Author String MA Bredella, E Lin, AV Gerweck, DJ Brick, LM Harrington, AL Utz, KK Miller
Massachusetts General Hospital, Boston, MA; Massachusetts General Hospital, Boston, MA; Vanderbilt University, Nashville, TN

Body **Context:** Visceral adiposity is associated with increased cardiovascular risk and decreased GH secretion. **Objective:** To determine whether administration of low dose GH in viscerally obese women improves body composition and cardiovascular risk markers. **Design:** 6-month, randomized, double-blind placebo controlled study. **Study participants:** 79 obese premenopausal women. **Main outcome measures:** Abdominal fat depots, including visceral adipose tissue (VAT) and muscle area of mid-thigh by CT, fat and lean mass by DXA, intramyocellular (IMCL) and intrahepatic (IHL) lipids by proton MR spectroscopy, hsCRP, total, HDL LDL cholesterol, apo B, fibrinogen, tPA, carotid intimal-medial thickness (IMT) and endothelial function. **Results:** The mean GH dose at 6 months was 1.7±0.6 mg/day, resulting in a mean IGF-1 SDS increase from -1.7±0.5 to -0.1±1.4 in the GH group. GH administration resulted in an increase in muscle area (2.0±5.4 vs. -3.0±6.8 cm², p=0.03) and total lean mass (2.0±1.7 vs. 0.1±2.1 kg, p=0.001), and a decrease in trunk-extremity fat ratio (0.01±0.05 vs. -0.03±0.06, p=0.006) compared with placebo. Change in IGF-1 level was associated with 6-month decrease in VAT (r= -0.56, p=0.002), suggesting that subjects with the greatest increases in IGF-1 levels had the greatest decreases in VAT. GH decreased hsCRP (-1.1±1.2 vs. 0.07±1.2 mg/L, p=0.01), apo B (-9.1±17.9 vs. 6.1±17.5 mg/dl, p=0.005), apo B/LDL (a measure of LDL size and atherogenicity) (-0.009±0.1 vs. 0.1±0.2, p=0.01), and tpA (-0.4±5.0 vs. 4.5±10.3 ng/ml, p=0.03) compared with placebo. Total, LDL, HDL cholesterol, fibrinogen, IMCL and IHL did not change compared with placebo, but IMCL increased compared with baseline in the GH group. There was no effect on IMT or endothelial function. GH increased fasting glucose (2.4±7.2 vs. -0.9±3.6 mg/dl) and 2-hour glucose (18.6±32.8 vs. -0.7±26.7 mg/dl) compared with placebo (p<0.05), with no difference in 2-hour glucose levels between groups at 6 months. Baseline fasting glucose predicted 6-month change in 120 min glucose within th GH group (r= 0.48, p= 0.01). Five subjects had a 120-min glucose greater than 200 mg/dl, one of whom was on placebo. No subjects had fasting glucose levels [ge]126 mg/dl. There were few additional side effects. **Conclusion:** GH replacement in viscerally obese premenopausal women has beneficial effects on markers of cardiovascular risk and body composition but is associated with a decrease in glucose tolerance in a minority of women.

Sources of Research Support: In part by National Institutes of Health Grants RO1 HL-077674, UL1 RR-025758, and K23 RR-23090.

Nothing to Disclose: MAB, EL, AVG, DJB, LMH, ALU, KKM

Pub # OR27-6
Session Information ORAL SESSION: CLINICAL - Growth Hormone & IGFs: Links to Cancer, Metabolism & Mortality (11:15 AM-12:45 PM)
Title Associations of Insulin-Like Growth Factor-I and Its Binding Proteins 1 and 3 with All-Cause and Cardiovascular Mortality in Older Men: The Health in Men Study
Author String BB Yeap, P Chubb, KA McCaul, KKY Ho, GJ Hankey, PE Norman, L Flicker
University of Western Australia, Perth, Australia; Fremantle Hospital, Fremantle, Australia; Fremantle Hospital, Fremantle, Australia; University of Western Australia, Perth, Australia; Princess Alexandra Hospital, Brisbane, Australia; University of Western Australia, Perth, Australia

Body
Context
Growth hormone secretion and levels of insulin-like growth factor-I (IGF-I) decline with age while ill-health increases. In the circulation, most IGF-I is bound to binding proteins. Whether differences in the levels of IGF-I and its binding proteins 1 and 3 (IGFBP1 and IGFBP3) determine health outcomes in ageing men remains controversial.

Objective

To examine associations of IGF-I, IGFBP1 and IGFBP3 with all-cause and cardiovascular mortality in older men.

Design, setting and participants

This was a prospective cohort study of community-dwelling men aged 70 years or more. Plasma collected at baseline (2001-4) was assayed for total IGF-I, IGFBP1 and IGFBP3.

Main outcome measures

Incidence and causes of death to 31 Dec 2008, ascertained using the Western Australian Data Linkage System. Cumulative mortality was plotted according to quintiles of IGF-I, IGFBP1 and IGFBP3. Cox regression analyses were performed with adjustment for conventional cardiovascular risk factors.

Results

Among 3,983 men followed for 5.2 years (median), 694 deaths occurred, 243 from cardiovascular disease (CVD). There was no difference in survival according to quintiles of IGF-I. Increased IGFBP1 predicted increased all-cause mortality (highest vs lowest quintile: adjusted hazard ratio [HR]=1.98, 95% confidence interval [CI]=1.52-2.57, $p<0.001$ for trend) and increased cardiovascular mortality (HR=3.42 [2.03-5.77], $p<0.001$ for trend). Decreased IGFBP3 predicted increased all-cause mortality (lowest vs highest quintile: HR=1.57, 95% CI=1.23-2.01, $p=0.007$ for trend). Associations of IGFBP1 and IGFBP3 with all-cause mortality were not attenuated by additional adjustment for IGF-I levels (highest vs lowest quintile IGFBP1: HR=2.02, 95% CI=1.55-2.63; lowest vs highest quintile IGFBP3 HR=1.85, 95% CI=1.38-2.47). Omission of men receiving insulin or all men with diabetes did not alter these results.

Conclusions

In older men, higher IGFBP1 and lower IGFBP3 levels predict overall and CVD-related mortality while IGF-I levels are not associated with mortality. Despite the reported association of reduced IGFBP1 with insulin resistance, higher IGFBP1 levels were a risk predictor for all-cause and CVD deaths. Reduced IGFBP3 may be a marker for, or contribute to, increased mortality risk distinct from its relationship to IGF-I. Further studies are needed to clarify the underlying mechanisms by which IGFBP1 and IGFBP3 levels are associated with mortality, and whether these are independent of IGF-I.

Sources of Research Support: Hormone assays were funded by Project Grant 513823 from the National Health and Medical Research Council of Australia (NHMRC). The Health In Men Study was funded by NHMRC Project Grants 279408, 379600 and 403963. BBY is recipient of a Clinical Investigator Award from the Sylvia and Charles Viertel Charitable Foundation, New South Wales, Australia. PEN is supported by NHMRC Practitioner Fellowship 458505.

Nothing to Disclose: BBY, PC, KAM, KKYH, GJH, PEN, LF

Pub # OR28-1

Session Information ORAL SESSION: CLINICAL - Long-Term Outcomes & Consequences of Pituitary Disease (11:15 AM-12:45 PM)

Title Tumor Recurrence and Enlargement in Patients with Craniopharyngioma with and without Growth Hormone Replacement Therapy during More Than 10 Years of Follow-Up

Author String DS Olsson, M Buchfelder, K Wiendieck, N Kremenevskaja, B-A Bengtsson, K-E Jakobsson, M Jarfelt, G Johannsson, AG Nilsson
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Body Most patients with treated craniopharyngioma (CP) are growth hormone (GH) deficient. GH replacement therapy (GHRT) may stimulate tumor growth and one of the concerns with long-term GHRT is the potential risk of tumor recurrence or tumor enlargement. The aim was therefore to study tumor progression in patients with CP on long-term GHRT. 56 consecutive CP patients included into an open prospective study on the effects of long-term GHRT with >3 years of GHRT were studied (57% men). The mean age at diagnosis was 25±16 and the average duration of GHRT was 13.6±5.0 yrs. The GHRT patients were sampled with 70 suitable CP control patients who had not received GHRT, with regard to follow-up (mean 13.4±7.8 yrs), gender (49% men), age at diagnosis (mean 32±17 yrs) and initial radiotherapy (RT). 54% of the GHRT patients and 33% of the control patients were treated with initial RT. The percentage of patients with a residual CP after their initial treatment was 29% for the GHRT group and 47% for the control group. The 10 and 15 yrs progression free survival rate (PFSR) for the entire population (126 patients) was 72% and 67%, respectively. In order to study the association between tumor progression and possible risk factors, a Cox regression was performed for the following factors (hazard ratio (HR) [95% CI for HR] p-value); initial RT (0.13 [0.05-0.33] <0.001), residual tumor (3.2 [1.6-6.2] <0.001), age at diagnosis (1.0 [1.0-1.0] 0.40) and gender (0.55 [0.28-1.1] 0.08). The 10 and 15 yrs PFSR were 88% and 79% for the GHRT group and 57% and 53% for the control group, respectively. Patients with a residual tumor after their initial treatment had a 5 yrs PFSR of 73% in the GHRT group (69% RT) and 76% in the control group (64% RT) (p-value: 0.58). When adding GHRT as a possible risk factor the Cox regression showed the following associations to tumor progression: GHRT (0.57 [0.26-1.3] 0.17), initial RT (0.16 [0.06-0.40] <0.001), residual tumor (2.6 [1.3-5.3] <0.01) and gender (0.57 [0.29-1.1] 0.10). Adjusted for the factors above, the 10 and 15 yrs PFSR were 85% and 72% for the GHRT group and 65% and 60% for the control group, respectively. This study reports new long-term data on tumor progression rate in CP patients. The most important prognostic factors for the PFSR are residual tumor and initial RT. GHRT was not a risk factor for tumor progression. Our results provide further support that long-term use of GHRT in hypopituitarism is safe in patients with a residual CP.

Disclosures: GJ: Speaker, Eli Lilly & Company; Clinical Researcher, Novo Nordisk; Clinical Researcher, Pfizer, Inc.; Advisory Group Member, Otsuka; Chief Scientific Officer, DuoCort Pharma; Speaker, MerckSerono. Nothing to Disclose: DSO, MB, KW, NK, B-AB, K-EJ, MJ, AGN

Pub # OR28-2

Session Information ORAL SESSION: CLINICAL - Long-Term Outcomes & Consequences of Pituitary Disease (11:15 AM-12:45 PM)

Title Initial Hypothalamic Involvement Is the Major Risk Factor for Impaired Prognosis and Quality of Life in Childhood Craniopharyngioma Regardless of Chosen Treatment Strategies -- Results of RANIOPHARYNGEOM 2000

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Body
Background: Hypothalamic obesity has major impact on prognosis and quality of life (QoL) in childhood craniopharyngioma. The pathogenic relevance of initial hypothalamic involvement versus treatment-related hypothalamic lesions is a matter of controversy.
Patients and methods: 120 patients were recruited prospectively during 2001 and 2007 and evaluated after 3 years of follow-up (**KRANIOPHARYNGEOM 2000**). Body mass index (BMI) and QoL at diagnosis and 36 months after diagnosis were analyzed based on reference assessment of tumour localization and post-surgical hypothalamic lesions using a standardized grading system (no, anterior, posterior involvement / lesion). Treatment was analyzed regarding neurosurgical strategy of 50 participating neurosurgical centres and the centre sizes. Based on patient load during the 6-year recruitment period, participating centres were categorized as small (1 patient / 5 yrs), middle (2-5 patients / 5 yrs) or large sized centres (<5 patients / 5 yrs).
Results: BMI standard deviation score (SDS) at diagnosis was similar in patients with or without hypothalamic involvement. Surgical lesions of anterior and posterior hypothalamic areas were associated with higher increases in BMI SDS during 36 months post-diagnosis compared to patients without or only anterior lesion (+1.8 BMISD, p=0.033, +2.1 BMISD; p=0.011), negatively impacting QoL in patients with posterior hypothalamic lesions. Surgical strategies varied between the 50 neurosurgical centres (3 large, 24 middle-sized, 23 small centres). Patients treated in small centres presented with a higher rate of hypothalamic involvement compared to middle and large-sized centres. Treatment in large centres was less radical, the rates of complete resection and hypothalamic surgical lesions lower than those of middle-sized and small centres. However, a multivariable analysis showed that pre-operative hypothalamic involvement was the only independent risk factor for severe obesity (p=0.002).
Conclusions: Radical neurosurgical strategies leading to posterior hypothalamic lesions are not recommended due to potential to exacerbate hypothalamic obesity and impaired QoL. Because our results show that initial hypothalamic tumor involvement has an apriori effect on the clinical course, our recommendations are based on recognizing childhood craniopharyngioma as a chronic disease requiring experienced multidisciplinary teams in order to provide the best lifetime QoL for the patient.

Sources of Research Support: Deutsche Kinderkrebsstiftung, Bonn, Germany.

Nothing to Disclose: HLM, UG, CT, AF, IZ, MW-M, TP, FP, NS, GC

Pub # OR28-3

Session Information ORAL SESSION: CLINICAL - Long-Term Outcomes & Consequences of Pituitary Disease (11:15 AM-12:45 PM)

Title Impaired Aerobic Exercise Adaptation in Children and Adolescents with Craniopharyngioma: Influence of Hypothalamic Involvement Rather Than Hormone Replacement

Author String X Piguél, P Abraham, F Gatelais, R Coutant
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Body Despite appropriate pituitary hormonal substitutive treatment, general quality of life is often impaired in patients treated for craniopharyngioma, especially in physical subscales. Most of the patients complain of fatigue, and reduction in physical activity.
We assessed exercise tolerance using a standardized high-intensity cycle ergometer test in 17 children with surgically treated craniopharyngioma and appropriate pituitary hormone replacement therapy (7 of whom had hypothalamic involvement), compared to 22 healthy controls similar for age, gender, height, body mass index (BMI), percent body fat, and weekly sport duration.
Mean age was 15.2 ± 3.3 years and 15.4 ± 1.8 years in 17 children with craniopharyngioma and the 22 controls, respectively, with no difference between boys and girls. Mean age, height SDS, BMI SDS, percent fat mass, fat free mass, as well as median weekly sport duration were similar between the two groups. VO_{2max} to body weight, VO_{2max} to fat free mass, and maximal systolic blood pressure (BP) increment were significantly lower in children with craniopharyngioma compared to controls: 29.0 ± 9.8 vs. 37.8 ± 10.9 ml/kg.min, 39.4 ± 9.8 vs. 49.5 ± 10.8 ml/kg.min, and 40 ± 17 vs. 67 ± 23 mm Hg, respectively ($p < 0.05$). VO_{2max} to body weight and VO_{2max} to fat free mass were comparable between children with ($n = 6$) and without ($n = 11$) rhGH treatment. Children with hypothalamic involvement ($n = 7$) had higher BMI SDS (2.1 ± 0.4 vs. 0.4 ± 0.8 SDS) and percent fat mass (32.2 ± 10.9 vs. 20.6 ± 3.9 %) than children without hypothalamic involvement ($n = 10$), and lower VO_{2max} to body weight and VO_{2max} to fat free mass: 24.3 ± 6.4 vs. 35.8 ± 10.1 ml/kg.min ($p < 0.05$), and 35.5 ± 6.6 vs. 45.0 ± 11.4 ml/kg.min ($p < 0.05$), respectively. In conclusion, we found that children and adolescents with surgically treated craniopharyngioma had a decrease in maximal systolic BP as well as in aerobic capacity during an exercise test despite adequate hormone substitution. This difference was strongly related to hypothalamic involvement.

Nothing to Disclose: XP, PA, FG, RC

Pub # OR28-4
Session Information ORAL SESSION: CLINICAL - Long-Term Outcomes & Consequences of Pituitary Disease (11:15 AM-12:45 PM)
Title Low Total Cortisol Correlates Closely with Low Free Cortisol in Traumatic Brain Injury and Predicts Mortality and Long-Term Hypopituitarism
Author String MJ Hannon, RK Crowley, LA Behan, EP O'Sullivan, B Rogers, MC O'Brien, D Rawluk, R O'Dwyer, A Agha CJ Thompson
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Body Published data has demonstrated that low 0900h plasma total cortisol (PTC) in the acute phase following traumatic brain injury (TBI) predicts mortality. However, there is concern regarding the use of PTC to evaluate the pituitary-adrenal axis in acutely unwell patients due to potential discrepancies between PTC and plasma free cortisol (PFC) due to variations in corticosteroid binding globulin (CBG). We hypothesised that low PTC would correlate closely with PFC and would predict mortality and long-term hypopituitarism. 100 patients (84 men, median age 33, range 18-75) were recruited on admission with TBI (mean GCS \pm SD = 8.59 \pm 4.2). Each patient had PTC and CBG measured on days 1, 3, 5, 7, and 10 following TBI. Results were compared with 15 patients admitted to ITU following vascular surgery. A PTC <300nmol/L in a patient in ITU was regarded clinically as inappropriately low. PFC was calculated for 25% of TBI samples and all control samples using Coolen's equation (1). TBI patients reattended for dynamic pituitary testing >6 months after TBI. All controls had PTC >500 nmol/L on day 1, and >300 nmol on days 3-10. By contrast, 78/100 TBI patients had at least one PTC <300 nmol/L. TBI patients in the lowest quartile of final PTC measurement had the highest mortality (p=0.0187). PTC correlated closely with PFC in both TBI patients (r=0.99, p<0.0001) and controls (r=0.99, p<0.0001). 32/79 (40.5%) of TBI survivors attended for dynamic pituitary testing. The median time to dynamic pituitary testing was 14 months (range 6-24 months). 15/32 (46.9%) underwent insulin tolerance testing, 9/32 (28.1%) underwent glucagon testing and 8/32 (25%) underwent short synacther testing. 6/32 (18.8%) were ACTH deficient, of whom 5/6 (83.3%) previously had low PTC. 6/32 were GH deficient, all of whom previously had low PTC. One patient was gonadotropin deficient; he previously had low PTC. No patients were TSH or prolactin deficient. Overall, 12/32 (37.5%) had one or more pituitary hormone deficits. Lower mean PTC and final PTC were strongly associated with the development of chronic hypopituitarism (p = 0.049 and 0.015 respectively). There were no significant differences between those patients with acute or chronic hypopituitarism and those with no deficits in terms of age, initial GCS or CT appearances. PTC is an accurate surrogate marker of PFC in acutely unwell patients. Low PTC following TBI is predictive of mortality, and long-term hypopituitarism in TBI survivors.

(1) Coolens JL, Van Baelen H, Heyns W. Clinical use of unbound plasma cortisol as calculated from total cortisol and corticosteroid-binding globulin. *J Steroid Biochem.* 1987 Feb;26(2):197-202.

Sources of Research Support: Grant from Novo Nordisk.

Nothing to Disclose: MJH, RKC, LAB, EPO, BR, MCO, DR, RO, AA, CJT

Pub # OR28-5

Session Information ORAL SESSION: CLINICAL - Long-Term Outcomes & Consequences of Pituitary Disease (11:15 AM-12:45 PM)

Title Incidence of Venous Thromboembolism in Patients with Cushing Syndrome: A Multicenter Cohort Study

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Body Background: Venous thrombosis has frequently been reported in patients with endogenous Cushing's syndrome (CS).
Objective: To evaluate the incidence of venous thromboembolism (VTE) in patients with CS prior to treatment onset and after surgery.
Design: Multicenter cohort study
Patients and methods: Medical records of all patients with endogenous CS of benign origin were reviewed among all 8 university medical centers in the Netherlands. All objectively confirmed VTE's during three years prior to, and three years after treatment onset, within the study period of January 1st, 1990 to June 6th, 2010, were documented. Patients surgically treated for non-functioning pituitary adenoma served as a control group for the incidence of post-operative VTE in adrenocorticotropic hormone (ACTH)-dependent CS. Incidences of VTE were expressed as incidence rates.
Results: A total of 473 patients with CS (mean age 42 years, 363 women) were included (360 with ACTH-dependent CS). The total number of person years was 2526. Thirty-seven patients experienced a VTE during the study period, resulting in an incidence rate of 14.6 (95% confidence interval [CI] 10.3-20.1) per 1000 person-years. The incidence rate for first-ever VTE prior to treatment was 12.9 (95% 7.5-12.6) per 1000 person-years (17 events). The risk of post-operative VTE, defined as risk within three months after surgery, was 0% for ACTH-independent and 3.4 (95% CI 2.0-5.9%) for ACTH-dependent CS (12 events in 350 patients); most events occurred between 1 week and 2 months after surgery. Compared to the control group, the incidence rate ratio was 4.6 (95% CI 1.4-15.1) for VTE in patients undergoing transsphenoidal surgery for ACTH-dependent CS.
Conclusions: Patients with Cushing's syndrome are at high risk of venous thromboembolism, especially during active disease and after surgery. General guidelines on the choice, intensity and duration of thromboprophylaxis are urgently needed.

Disclosures: WWdH: Advisory Group Member, Novartis Pharmaceuticals; Ipsen. Nothing to Disclose: DJFS, BEAvZ, JEAD, SCC, RAF, MAW, ARH, GEAvdB, MNK, PMJZ, EAAF, NEAS, MLD, AMP, OMD, VEAG

Pub # OR28-6

Session Information ORAL SESSION: CLINICAL - Long-Term Outcomes & Consequences of Pituitary Disease (11:15 AM-12:45 PM)

Title Long-Term Outcomes in Treated Cushing Disease

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Body Background: Cushing's Disease (CD) confers a fourfold excess mortality and is associated with significant morbidity. Recent data suggest continued cardiovascular risk five years after endocrine cure. However, whether risk persists despite disease remission has not been fully explored, nor have predictors of morbidity and mortality in treated CD patients.

Objective: To describe long term outcomes in patients with treated CD. Specifically, to characterize mortality and predictors of mortality, including metabolic and cardiovascular risk, after long-term remission.

Methods: A retrospective chart review evaluating 183 patients (38 men, 145 women, mean age at diagnosis 38.0 +/- 13.5) was conducted and a database identifying age at diagnosis, duration of exposure to excess cortisol, co-morbidities at diagnosis, treatment modalities and laboratory values was created. 30 patients were enrolled in a telephone questionnaire aimed at gathering follow up data including remission status and co-morbidities at follow up. Cox proportional hazards models were constructed to identify predictors of mortality and cardiovascular outcomes. McNemar's Chi-square test was used to compare patient co-morbidities and body composition at diagnosis and follow-up.

Results: The mean follow up for patients in the chart review was 107.6 +/- 83.5 mos. Longer exposure to excess cortisol significantly predicted increased mortality and increased number of vascular events (p=.004, p=.006). Older age at diagnosis also significantly predicted increased mortality (p=<.001). When evaluating 26 of the 30 patients still in remission at long term follow up (mean yrs in remission 12.2 +/- 5.3), there was no significant difference in co-morbidities including hypertension, diabetes, hyperlipidemia, and osteoporosis at diagnosis versus at follow up. Similarly, there was no significant difference in BMI and weight at diagnosis and follow up.

Conclusions: In CD, longer duration of glucocorticoid exposure is associated with increased mortality and vascular events even after long-term biochemical remission. Similarly, co-morbidities including hypertension and excess weight may persist. Further studies are warranted to explore long term outcomes in treated CD.

Nothing to Disclose: SF, JK, KDP, EBG

Pub # OR29-1

Session Information ORAL SESSION: CLINICAL - Metabolic Bone Disease: Clinical Trials (11:15 AM-12:45 PM)

Title Denosumab Therapy in Postmenopausal Women with Osteoporosis: Results from the First Two Years of the Freedom Trial Extension

Author String HG Bone, R Chapurlat, ML Brandi, JP Brown, E Czerwinski, NS Daizadeh, A Grauer, M-A Krieg, Z Man, D Mellstrom, S Radominski, J-Y Reginster, H Resch, JA Roman, C Roux, SR Cummings, C Libanati, S Papapoulos
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Body Denosumab (DMAb) is an approved therapy for postmenopausal women with osteoporosis at increased risk for fracture. The favorable risk/benefit of DMAb was shown in the pivotal, placebo-controlled, 3-year FREEDOM trial. All women who completed FREEDOM were invited to participate in an extension study to monitor DMAb safety and efficacy for up to 10 years.

In the extension, all women receive 60 mg DMAb every 6 months. For the FREEDOM placebo group, the data here reflect up to 4 DMAb doses (2 years; cross-over group). For the FREEDOM DMAb group, the data reflect up to 10 DMAb doses (5 years; long-term group). The efficacy and general safety long-term data were previously presented. Here, we report the cross-over data and additional safety data.

Of those who completed FREEDOM, 4550 (70%) enrolled in the extension (2207 cross-over; 2343 long-term). During the first 2 years of DMAb, the cross-over group had significant ($P<0.0001$) gains in lumbar spine (7.9%) and total hip (4.1%) BMD, similar to those in the DMAb group in the first 2 years of FREEDOM. In the long-term group, further significant ($P<0.0001$) increases in BMD occurred for cumulative 5-year gains of 13.7% (lumbar spine) and 7.0% (total hip). CTX was rapidly and similarly reduced after the 1st (cross-over) or 7th (long-term) DMAb dose with the characteristic attenuation of this effect at the end of the dosing period. In the cross-over group, yearly incidences of new vertebral and nonvertebral fractures were lower than in the FREEDOM placebo group. Fracture incidence remained low in the long-term group. Incidences of adverse events (AEs) and serious AEs (SAEs) in the cross-over group were similar to or lower than in the placebo and DMAb FREEDOM groups, and AEs or SAEs did not increase over time with long-term DMAb. In particular, subject incidence rates of SAEs of infection in years 4 and 5 of DMAb were similar to or lower than yearly rates in the FREEDOM placebo group. This was also the case for individual SAEs of infection including pneumonia, urinary tract infection, diverticulitis, gastroenteritis, cellulitis/erysipelas, and bronchopneumonia. Two oral AEs were adjudicated to ONJ in the cross-over group and none in the long-term group; both healed completely and 1 woman continued DMAb. No atypical fractures occurred.

The FREEDOM extension study safety and efficacy results are consistent with the original FREEDOM study observations and provide long-term exposure data for up to 5 years in 2343 women.

Sources of Research Support: Amgen Inc.

Disclosures: HGB: Investigator, Amgen; Merck & Co.; Nordic Bioscience; Advisory Group Member, Amgen Merck & Co.; Novartis Pharmaceuticals; Honoraria, Amgen; Merck & Co.; Speaker Bureau Member, Amgen RC: Investigator, Servier; Merck & Co.; Sanofi-Aventis; Novartis Pharmaceuticals; Warner Chilcott; Consultant, Servier; Novartis Pharmaceuticals; Amgen; Merck & Co. MLB: Speaker, Amgen; Merck & Co.; Servier; Warner Chilcott; GlaxoSmithKline; Eli Lilly & Company. JPB: Coinvestigator, Abbott Laboratories; Roche Pharmaceuticals; Principal Investigator, Amgen; Eli Lilly & Company; Merck & Co.; Novartis Pharmaceuticals; Pfizer, Inc.; Sanofi-Aventis; Serono; Servier; Warner Chilcott; Advisory Group Member, Amgen; Eli Lilly & Company; Novartis Pharmaceuticals; Warner Chilcott; Speaker, Amgen; Eli Lilly & Company; Merck & Co.; Novartis Pharmaceuticals; Roche Pharmaceuticals; Consultant, Sanofi-Aventis. EC: Investigator, Eli Lilly & Company; Novartis Pharmaceuticals; Roche Pharmaceuticals; Amgen; Pfizer, Inc.; Servier; Merck & Co.; Serono; Astra Zeneca; Merck Sharp & Dohme; SantoSolve AS; Danone Research; Speaker, Servier; Roche Pharmaceuticals. NSD: Employee, Amgen. AG: Employee, Amgen. ZM: Principal Investigator, Amgen; Astra Zeneca; Bayer, Inc.; Eli Lilly & Company; Merck & Co.; Novartis Pharmaceuticals; NPS Allelix; Proctor & Gamble; Aventis Pharmaceuticals; Sanofi-Aventis; Roche Pharmaceuticals; Wyeth Pharmaceuticals; Medical Advisory Board Member, GlaxoSmithKline; Sanofi-Aventis; Speaker, Novartis Pharmaceuticals; Sanofi-Aventis; Roche Pharmaceuticals; Committee Member, Novartis Pharmaceuticals. SR: Principal Investigator, Amgen; Speaker, Eli Lilly & Company; Principal Investigator, Novartis Pharmaceuticals; Principal Investigator, Pfizer, Inc.; Principal Investigator, Roche Pharmaceuticals; Speaker Bureau Member, Sanofi-Aventis. J-YR: Consultant, Servier; Novartis Pharmaceuticals; Negma; Eli Lilly & Company; Wyeth Pharmaceuticals; Amgen; GlaxoSmithKline; Roche

Pharmaceuticals; Merck & Co.; Nycomed; NPS; Theramex; UCB; Speaker, Merck Sharp & Dohme; Eli Lilly & Company ; Rottapharm; IBSA; Speaker, Genevrier; Novartis Pharmaceuticals; Servier; Roche Pharmaceuticals; GlaxoSmithKline; Teijin; Teva; Ebewee Pharma; Zodiac; Analis; Thermamex; Nycomed; Novo Nordisk; Investigator, Bristol-Myers Squibb; Merck Sharp & Dohme; Rottapharm; Teva; Eli Lilly & Company; Novartis Pharmaceuticals; Roche Pharmaceuticals; GlaxoSmithKline; Amgen; Servier. CR: Investigator, Amgen; Consultant, Amgen; Speaker, Amgen. SRC: Speaker, Amgen; Consultant, Amgen. CL: Employee, Amgen; Employee, Amgen. SP: Consultant, Amgen; Merck & Co.; Novartis Pharmaceuticals; Eli Lilly & Company; Proctor & Gamble; Consultant, GlaxoSmithKline. Nothing to Disclose: M-AK, DM, HR, JAR

Pub # OR29-2

Session Information ORAL SESSION: CLINICAL - Metabolic Bone Disease: Clinical Trials (11:15 AM-12:45 PM)

Title Physiological Estrogen Replacement Increases Bone Density in Adolescent Girls with Anorexia Nervosa

Author String M Misra, D Katzman, KK Miller, N Mendes, M Russell, D Snelgrove, DB Herzog, A Klibanski
Massachusetts General Hospital and Harvard Medical School, Boston, MA; Mass General Hospital for Children and Harvard Medical School, Boston, MA; Hospital for Sick Children, Toronto, Canada; Massachusetts General Hospital, Boston, MA

Body

Background: Anorexia nervosa (AN) is prevalent in adolescents and is associated with decreased bone accrual rates and low bone mineral density (BMD) at a time critical for optimizing peak bone mass. Low BMD, typically most severe at the lumbar spine, is consequent to multiple nutritional and hormonal alteration including hypogonadism and low IGF-1. Although recovery is associated with some improvement in BMD, residual deficits persist. Therapeutic strategies that increase BMD in AN are limited. Specifically, high doses of oral estrogen given as an oral contraceptive do not improve BMD in AN, possibly from IGF-1 suppression by oral estrogen. However, the impact of physiological doses of oral estrogen to mimic early puberty and of transdermal estrogen (which does not suppress IGF-1) on bone accrual has not been examined.

Subjects and Methods: We enrolled 110 girls with AN and 40 normal-weight controls (C) 12-18y of similar pubertal stage and bone age; subjects were studied for 18 mo. Immature AN [bone age (BA) <15y; n=14] were randomized to receive low dose oral ethinyl estradiol (3.75mcg daily from 0-6m, 7.5mcg from 6-12m and 11.25mcg from 12-18m) or placebo, while mature AN (BA[ge]15 y; n=96) were randomized to replacement transdermal estradiol (100mcg 17 β estradiol) (with cyclic progesterone) or placebo for 18m. Controls were followed without intervention for 18m. All received calcium and vitamin D. We used DXA to assess BMD at the lumbar spine (L), hip and whole body (WB). Height adjusted measures of BMD, i.e. spine bone mineral apparent density (BMAD) and WB bone mineral content/height (BMC/Ht) were calculated. Our primary end point was the change in LBMD over time.

Results: All BMD measures were lower in AN than C. AN randomized to estrogen (AN E+) did not differ from those randomized to placebo (AN E-) for age, BA, height, BMI, amenorrhea duration and BMD parameters. Changes in LBMD, LBMAD and hip BMD measures at 6, 12 and 18m, and WB BMC/Ht at 6 and 12m were significantly lower in AN E- vs. C (p<0.05) but were comparable in AN E+ vs. C. BMD changes were predicted inversely by baseline age and positively by weight changes. AN E+ compared with AN E- had greater increases in LBMD and LBMAD at 6, 12 and 18m (p<0.05) and in hip BMD at 18m (p<0.05) even after controlling for baseline age and weight changes.

Conclusion: Physiological estradiol replacement in adolescent girls with AN is effective in optimizing bone accrual rates to approximate that in controls.

Sources of Research Support: 1 UL1 RR025758-01, R01 DK 062249, K23 RR018851.

Nothing to Disclose: MM, DK, KKM, NM, MR, DS, DBH, AK

Pub # OR29-3

Session Information ORAL SESSION: CLINICAL - Metabolic Bone Disease: Clinical Trials (11:15 AM-12:45 PM)

Title A Randomized Controlled Trial of Annual Low-Dose Zoledronate in Postmenopausal Women

Author String A Grey, M Bolland, S Wong, A Horne, G Gamble, IR Reid
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Body Annual administration of 5mg of intravenous zoledronate (Zol), which reduces risk of fracture by 35-70%, and decreases mortality by 28% after hip fracture, is widely used for treatment of osteoporosis. However, the optimal dosing regimen of Zol is not known. In the 12 month phase II Zol trial, several dosing regimens were equally effective in decreasing markers of bone turnover and increasing bone mineral density (BMD), including those that involved administration of a total dose of 2mg, given as 0.5mg every 3 months, and of a total dose of 1mg, given as 0.25mg every 3 months. The anti-resorptive effects of *annual* doses of Zol below 4mg have not been studied. To address this gap in knowledge, we conducted a randomized trial in 180 osteopenic postmenopausal women, who were allocated to receive a single dose of placebo or 1, 2.5 or 5mg of intravenous Zol. The primary endpoint was change in lumbar spine BMD at 12 months. Secondary endpoints were change in total hip BMD, and changes in markers of bone turnover. After 12 months, change in spine BMD was greater in each of the Zol groups than the placebo group [mean (95% CI) Zol 1mg 3.5% (2.2, 4.8); Zol 2.5mg 4.0% (2.7, 5.3); Zol 5mg 3.6% (2.3, 4.9), P<0.001 vs placebo for each Zol dose]. Change in BMD at the total hip was also significantly greater in each of the Zol groups than the placebo group [Zol 1mg 2.7% (1.9, 3.5); Zol 2.5mg 3.6% (2.8, 4.4); Zol 5mg 3.6% (2.8, 4.4), P<0.001 vs placebo for each Zol dose]. Each of the turnover markers, β -CTX and P1NP, decreased significantly in each of the Zol groups [β -CTX, Zol 1mg -44% (-55, -33); Zol 2.5mg -68% (-78, -57); Zol 5mg -73% (-83, -63); P1NP, Zol 1mg -41% (-55, -28); Zol 2.5mg -58% (-72, -44); Zol 5mg -64% (-78, -51), P<0.001 vs placebo for each marker for each Zol dose]. Markers of bone turnover in each Zol group were stably decreased between 6 and 12 months. Acute phase reaction tended to occur less frequently in the 1mg Zol group than in either of the higher dose groups. These data (a) demonstrate that annual administration of doses of Zol of either 1 or 2.5mg produces substantial anti-resorptive effects that approximate those of the 5mg dose, and (b) suggest that such doses may confer significant anti-fracture efficacy.

Sources of Research Support: Health Research Council of New Zealand.

Disclosures: IRR: Advisory Group Member, Novartis Pharmaceuticals; Investigator, Novartis Pharmaceuticals; Speaker, Novartis Pharmaceuticals. Nothing to Disclose: AG, MB, SW, AH, GG

Pub # OR29-4
Session Information ORAL SESSION: CLINICAL - Metabolic Bone Disease: Clinical Trials (11:15 AM-12:45 PM)
Title Denosumab Decreases Cortical Porosity in Postmenopausal Women with Low BMD
Author String SK Boyd, KK Nishiyama, RM Zebaze, DA Hanley, JR Zanchetta, T Thomas, S Boutroy, C Bogado, M Austin, C Libanati, E Seeman
University of Calgary, Calgary, Canada; University of Melbourne, Melbourne, Australia; Instituto de Investigaciones Metabolicas, Buenos Aires, Argentina; University Hospital of St-Etienne, St-Etienne, France; Universite de Lyon, Lyon, France; Amgen Inc, Thousand Oaks, CA; University of Melbourne, Melbourne, Australia; University of Calgary, Calgary, Canada

Body Intracortical remodeling, particularly in cortex adjacent to the marrow cavity, is responsible for most of the bone lost during advancing age (1,2). Indeed, patients who suffer a hip fracture have large coalescent pores that precipitously compromise bone strength as porosity increases (3). Denosumab reduces bone remodeling and reduces ovariectomy-induced intracortical porosity in subhuman primates. In postmenopausal women, denosumab rapidly reduces bone remodeling intensity and increases cortical density (4-6). We propose that this increase in cortical density is the result of (i) partial reversal of intracortical porosity that is present prior to treatment, (ii) reduced appearance of new pores by suppressing remodeling and (iii) more complete secondary mineralization of osteons (which would be removed had remodeling continued at the same intensity). We now present evidence to support several of these mechanisms.
Postmenopausal women (N=247) aged 61 ± 5 yrs with low bone mineral density were randomized in a double-blind, double-dummy fashion to denosumab 60 mg Q6M (N=83), alendronate 70 mg QW (N=82), or placebo (N=82). Porosity was evaluated in the compact appearing cortex of the distal radius at baseline and month 12 from HRpQCT scans using an enhanced method that accurately (7) and reproducibly (8) identifies the cortex with automatic threshold segmentation (9). Pores above ~ 82 [micro]m are quantifiable; porosity is expressed as a percent of the total cortical volume.
Baseline cortical porosity at the distal radius was 2.6%. Over 12 months, cortical porosity (mean [95% CI]) increased with placebo (5.19% [1.41, 8.98]) and alendronate (2.86% [-2.32, 8.04]) but decreased with denosumab (2.98% [-6.39, 0.43]). Denosumab significantly reduced cortical porosity by 8.18% (P=0.01) compared with placebo.
In summary, denosumab prevented the progression of porosity seen with placebo, an effect that differs from that of alendronate. The changes in porosity are likely to partly reverse bone fragility and prevent its progression and thus reduce fracture risk. Ongoing work exploring non-threshold methods and assessing porosity over the entire cortex including the trabecularized cortex is underway and may improve quantification of bone morphology and differences between therapies.

- (1) Zebaze et al, Lancet 2010;375:1729-36
- (2) Holzer et al, JBMR 2009;24:468-74
- (3) Bell et al, Bone 2000;27:297-304
- (4) Seeman et al, JBMR 2010;25:1886-94
- (5) Genant et al, Bone 2010;47:131-39
- (6) Baron et al, Bone 2010; epub ahead of print
- (7) Nishiyama et al, JBMR 2010;25:882-90
- (8) Burghardt et al, Bone 2010;47:519-28
- (9) Buie et al, Bone 2007;41:505-15

Sources of Research Support: Amgen Inc.

Disclosures: SKB: Researcher, Amgen; Servier; Consultant, Merck & Co. RMZ: Coinvestigator, Amgen; Speaker, Servier. DAH: Clinical Researcher, Amgen; Eli Lilly & Company; NPS; Servier; Merck & Co.; Novartis Pharmaceuticals; Warner-Chilcott; Medical Advisory Board Member, Amgen; Eli Lilly & Company Novartis Pharmaceuticals; Warner-Chilcott ; Speaker, Amgen; Eli Lilly & Company; Novartis Pharmaceuticals; Merck & Co.; Warner-Chillcott. JRZ: Investigator, Amgen; Eli Lilly & Company; Merck & Co.; Pfizer, Inc.; Medical Advisory Board Member, Amgen; Eli Lilly & Company; GlaxoSmithKline; Merck & Co.; Pfizer, Inc.; Servier; Consultant, Amgen; Eli Lilly & Company; GlaxoSmithKline; Merck & Co.; Pfizer, Inc.; Servier. TT: Investigator, Amgen; Chugai; Eli Lilly & Company; Merck & Co.; Pfizer, Inc.; Roche Pharmaceuticals; Servier; Warner-Chilcott; Medical Advisory Board Member, Amgen; Merck & Co.; Novartis Pharmaceuticals; Consultant, Daiichi-Sankyo; Bristol-Myers Squibb; Eli Lilly & Company; Speaker Amgen; Daiichi-Sankyo; GlaxoSmithKline; Eli Lilly & Company; Merck & Co.; Novartis Pharmaceuticals; Roche Pharmaceuticals; Servier; Warner-Chilcott. CB: Medical Advisory Board Member, GlaxoSmithKline. MA: Employee, Amgen. CL: Employee, Amgen. ES: Speaker Bureau Member, Amgen; Servier; Sanofi-Aventis; MSD; Novartis Pharmaceuticals; Medical Advisory Board Member, Amgen; Servier; Sanofi-Aventis; MSD; Novartis Pharmaceuticals; Consultant, Amgen; Servier; Sanofi-Aventis; MSD; Novartis Pharmaceuticals. Nothing to Disclose: KKN, SB

Pub # OR29-5

Session Information ORAL SESSION: CLINICAL - Metabolic Bone Disease: Clinical Trials (11:15 AM-12:45 PM)

Title Three-Year Experience of Continuous Subcutaneous Recombinant PTH (1-34) Replacement Therapy in Three Children with Hypoparathyroidism

Author String A Rothenbuhler, I Gueorguieva, P Luccini, A Linglart, P Bougneres
University Paris-Descartes, H[ocirc]pital St Vincent de Paul, Paris, France

Body Hypoparathyroidism in adults and children is commonly treated with calcium and Vitamin D supplements which correct hypocalcemia by forcing intestinal calcium absorption and thus replaces the renal failure to produce 1,25 dihydroxyvitamin D. Repeated hypocalcemic seizures alternating with periods of hypercalcemia/hypercalciuria under conventional treatment with 1- α -calcidiol lead us to treat two children (8 and 13 year old boys), one with APECED, hypoparathyroidism and digestive malabsorption; one with idiopathic hypoparathyroidism, with continuous subcutaneous recombinant PTH (1-34) (rhPTH¹⁻³⁴) administered via an insulin pump during three years. The third patient is the 11 year old sibling of the patient with APECED in whom continuous rhPTH¹⁻³⁴ was chosen as first line therapy. The dose of rhPTH¹⁻³⁴ necessary to maintain ionized calcium levels between 1.18 and 1.30 mmol/l were between 5 and 30 [micro]g/day. Maintenance of 25-OH vitamin D levels above 40 ng/ml allowed a four-fold decrease of the initial rhPTH¹⁻³⁴ dose. A significant decrease in the number of hypocalcemic seizures, emergency hospitalisations, IV calcium loads and blood draws per year was observed after rhPTH¹⁻³⁴ pump initiation and urinary calcium excretion also decreased significantly. Substitutive continuous rhPTH¹⁻³⁴ is an efficient treatment in these three children two of which had refractory hypoparathyroidism.

Nothing to Disclose: AR, IG, PL, AL, PB

Pub # OR29-6

Session Information ORAL SESSION: CLINICAL - Metabolic Bone Disease: Clinical Trials (11:15 AM-12:45 PM)

Title Vitamin D, OPG/RANKL, and Inflammation with Alendronate Treatment in HIV-Infected Patients with Reduced Bone Mineral Density: A Secondary Analysis of AIDS Clinical Trial Group (ACTG) A5163

Author String T Brown, M Kendall, G McComsey
Johns Hopkins University, Baltimore, MD; Harvard School of Public Health, Boston, MA; Case School of Medicine, Cleveland, OH

Body **Background:** Chronic HIV infection is an inflammatory disease associated with osteoporosis. As in other populations, bisphosphonates are the first-line treatment in HIV+ persons, but their mechanism of action is not completely understood. Changes in the osteoprotegerin (OPG)/receptor activator for nuclear factor kappa B ligand (RANKL) system and systemic inflammation have been proposed as additional mechanisms for the observed treatment effect. We undertook a secondary analysis of a randomized, placebo-controlled study in HIV+ persons with low BMD (ACTG A5163) to: 1) determine the effect of alendronate (ALN) on inflammatory markers and OPG/RANKL, and 2) explore the effect of systemic inflammation and other baseline factors on the BMD response to ALN.

Methods: Eighty-two HIV+ patients with lumbar spine T-score [le] -1.5 were randomized to ALN 70 mg weekly or placebo for 48 weeks. All subjects received calcium carbonate 500 mg/Vitamin D3 200 IU twice daily. Subjects with baseline 25 hydroxyvitamin D < 15 ng/mL were excluded. C-telopeptides (CTx) and BMD were assessed at baseline, week 24 and week 48. Stored plasma samples were assayed for OPG, RANKL, IL-6, and soluble receptors for TNF-alpha 1 & 2 (sTNFR 1 & 2).

Results: 70 subjects (36 ALN; 34 placebo) had complete data at 48 weeks. ALN was associated with greater mean (\pm SD) increases in BMD than placebo at the hip ($3.8 \pm 4.2\%$ vs $1.5 \pm 3.6\%$, $p=0.02$) and the spine ($4.0 \pm 4.9\%$ vs $1.3 \pm 3.6\%$, $p=0.01$). No within- or between-arm differences in OPG, RANKL or inflammatory markers were observed over 48 weeks. Those with the highest baseline sTNFR2 concentrations (top 50%) had the most robust effect of ALN at the total hip at 48 weeks (difference ALN-placebo, 5.0% vs -0.5% , $p=0.003$) whereas those with the highest CTx concentrations at baseline had a more pronounced ALN effect at the lumbar spine (difference ALN-placebo, 5.7% vs -0.6% , $p=0.001$). Baseline 25OHD < 32 ng/mL was associated with larger increases in hip BMD over 48 weeks, independent of ALN treatment ($p=0.01$).

Conclusions: Among HIV+ patients, higher baseline TNF-alpha activity and bone resorption were associated with an increased effect of alendronate at the hip and spine, respectively. The association between low vitamin D levels and BMD response, independent of alendronate, reinforces the importance of concomitant vitamin D and calcium supplementation with bisphosphonate treatment.

Sources of Research Support: Merck; AIDS Clinical Trials Group.

Nothing to Disclose: TB, MK, GM

Pub #	CP1
Session Information	CLINICAL PRACTICE GUIDELINE: CLINICAL - Evaluation, Treatment & Prevention of Vitamin D Deficiency: An Endocrine Society Clinical Practice Guideline (11:15 AM - 12:00 PM)
Title	Moderator
Author String	MJ Favus University of Chicago Medical Center, Chicago, IL
Body	

Pub # CDW5

Session Information CAREER DEVELOPMENT WORKSHOP: Career Development Workshop: Negotiations -- Basic & Clinical Research (11:15 AM-12:15 PM)

Title Negotiations: Basic & Clinical Research

Author String DJ Good
Virginia Polytechnic Institute and State University, Blacksburg, VA

Body Many job seekers accept the first offer they receive, and later regret not asking for specific things, such as increased pay, technician assistance, needed equipment and even moving costs, all of which could have helped them to better succeed in their new position. Deborah J. Good, an Associate Professor at Virginia Tech, has served on numerous job search committees, and has negotiated several different jobs and job offers --some better than others! This workshop will use the speaker's personal experience to examine some of the dos and don'ts of job offer negotiations. Job seekers at the assistant and associate professor level, as well as graduate students looking towards their first postdoctoral fellowship, are welcome to attend and bring their questions to this interactive workshop.

Nothing to Disclose: DJG

Pub #	PMW1-1
Session Information	WORKSHOP: CLINICAL - How To Negotiate with Your Health System for Resources: An Academic Practice Management Workshop (12:15 PM - 1:00 PM)
Title	How To Negotiate with Your Health System for Resources
Author String	JV Hennessey Beth Israel Deaconess Medical School, Boston, MA
Body	Nothing to Disclose: JVH

Pub # PMW1-2
Session Information WORKSHOP: CLINICAL - How To Negotiate with Your Health System for Resources: An Academic Practice Management Workshop (12:15 PM - 1:00 PM)
Title How To Negotiate with Your Health System for Resources
Author String MH Schutta
University of Pennsylvania's Penn Rodebaugh Diabetes Center, Philadelphia, PA
Body Disclosure Incomplete: MHS

Pub #	CDW6
Session Information	CAREER DEVELOPMENT WORKSHOP: Career Development Workshop: Negotiations -- Clinical Practice (12:30 PM - 1:00 PM)
Title	Negotiations: Clinical Practice
Author String	JA Wexler Washington Hospital Center, Silver Spring, MD
Body	Nothing to Disclose: JAW

Pub #	Y3
Session Information	ENDOCRINE YEAR IN BASIC/CLINICAL SCIENCE SESSION: BASIC - The Year in Orphan Nuclear Receptors (1:00 PM - 1:45 PM)
Title	The Year in Orphan Nuclear Receptors
Author String	DD Moore Baylor College of Medicine, Houston, TX
Body	Nothing to Disclose: DDM

Pub # CMF8-1
Session Information CASE MANAGEMENT FORUM: CLINICAL - Investigation & Management of Gynecomastia (1:00 PM - 1:45 PM)
Title Investigation & Management of Gynecomastia
Author String GD Braunstein
Cedars-Sinai Medical Center, Los Angeles, CA
Body Disclosures: GDB: Consultant, Abbott Laboratories, Esoterix (LabCorp); Principal Investigator, BioSante.

Pub #	CMF8-2
Session Information	CASE MANAGEMENT FORUM: CLINICAL - Investigation & Management of Gynecomastia (1:00 PM - 1:45 PM)
Title	Investigation & Management of Gynecomastia
Author String	BB Yeap University of Western Australia, Fremantle, Australia
Body	Nothing to Disclose: BBY

Pub #	M29
Session Information	MEET-THE-PROFESSOR: CLINICAL - Cases from the Metabolic Bone Clinic (1:00 PM - 1:45 PM)
Title	Cases from the Metabolic Bone Clinic
Author String	M Kleerekoper Wayne State University, Bloomfield Hills, MI
Body	Disclosures: MK: Speaker Bureau Member, Amgen, Eli Lilly & Company; Advisory Group Member, Roche Diagnostics, Ortho McNeil.

Pub # M30
Session Information MEET-THE-PROFESSOR: CLINICAL - Diagnostic Strategy for Post-Menopausal Androgen Excess (1:00 PM - 1:45 PM)
Title Diagnostic Strategy for Post-Menopausal Androgen Excess
Author String ME Wierman
Veterans Affairs Medical Center, Endocrine 111H, Denver, CO
Body Disclosure Incomplete: MEW

Pub #	M31
Session Information	MEET-THE-PROFESSOR: CLINICAL - Dopamine Agonists: When To Use, What To Choose & Safety Issues (1:00 PM - 1:45 PM)
Title	Dopamine Agonists: When To Use, What To Choose & Safety Issues
Author String	JA Romijn Academic Medical Center, Amsterdam, Netherlands
Body	Nothing to Disclose: JAR

Pub #	M32
Session Information	MEET-THE-PROFESSOR: CLINICAL - Management of Hormone Replacement in Addison Disease (1:00 PM - 1:45 PM)
Title	Management of Hormone Replacement in Addison Disease
Author String	BM Arafah Case Western Reserve University, Cleveland, OH
Body	Nothing to Disclose: BMA

Pub #	M33
Session Information	MEET-THE-PROFESSOR: CLINICAL - Management of Hypogonadotropic Hypogonadism (1:00 PM - 1:45 PM)
Title	Management of Hypogonadotropic Hypogonadism
Author String	AC Latronico Sao Paulo University, Sao Paulo, Brazil
Body	Nothing to Disclose: ACL

Pub # M34
Session Information MEET-THE-PROFESSOR: CLINICAL - Management of Idiopathic Short Stature (1:00 PM - 1:45 PM)
Title Management of Idiopathic Short Stature
Author String SD Chernausek
Oklahoma Health Sciences Center, Oklahoma City, OK
Body Session supported by: Lilly USA, LLC, Novo Nordisk Inc. & Pfizer, Inc.
Disclosure Incomplete: SDC

Pub # CMF6-1

Session Information CASE MANAGEMENT FORUM: CLINICAL - AACE vs ADA Guidelines for Diagnosis & Management of Type 2 Diabetes Mellitus (1:00 PM - 1:45 PM)

Title AACE vs ADA Guidelines for Diagnosis & Management of Type 2 Diabetes Mellitus

Author String JA Davidson
University of Texas Southwestern Medical Center, Dallas, TX

Body Session supported by: Amylin Pharmaceuticals, Inc., Lilly USA, LLC and Merck & Co., Inc.

Disclosure Incomplete: JAD

Pub # CMF6-2

Session Information CASE MANAGEMENT FORUM: CLINICAL - AACE vs ADA Guidelines for Diagnosis & Management of Type 2 Diabetes Mellitus (1:00 PM - 1:45 PM)

Title AACE vs ADA Guidelines for Diagnosis & Management of Type 2 Diabetes Mellitus

Author String DM Nathan
Massachusetts General Hospital/Harvard Medical School, West Newton, MA

Body Session supported by: Amylin Pharmaceuticals, Inc., Lilly USA, LLC and Merck & Co., Inc.

Nothing to Disclose: DMN

Pub #	CMF7-1
Session Information	CASE MANAGEMENT FORUM: CLINICAL - Diagnosis of Thyroid Cancer Using Ultrasound & Fine Needle Aspiration (1:00 PM - 1:45 PM)
Title	Diagnosis of Thyroid Cancer Using Ultrasound & Fine Needle Aspiration
Author String	B McIver Mayo Clinic, Rochester, MN
Body	Nothing to Disclose: BM

Pub #	CMF7-2
Session Information	CASE MANAGEMENT FORUM: CLINICAL - Diagnosis of Thyroid Cancer Using Ultrasound & Fine Needle Aspiration (1:00 PM - 1:45 PM)
Title	Diagnosis of Thyroid Cancer Using Ultrasound & Fine Needle Aspiration
Author String	RT Kloos Ohio State University, Columbus, OH
Body	Disclosures: RTK: Study Investigator, Veracyte, Inc.

Pub # P3-1

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Actions of Adrenal Steroid Receptors & Other Nuclear Receptors (1:30 PM-3:30 PM)

Title Are Glucocorticoids Essential for Proper Cardiac Maturation *In Utero*?

Author String EA Rog-Zielinska, A Thomson, C Moran, DG Brownstein, CJ Kenyon, Z Michailidou, D Szumska, S Bhattacharya, J Richardson, E Owen, L Forrester, MC Homes, KE Chapman, B Walker
University of Edinburgh, Edinburgh, UK; University of Oxford, Oxford, UK

Body Glucocorticoid levels rise dramatically in late gestation in mammals and are essential for organ maturation in preparation for birth. They are widely used clinically to accelerate the lung maturation of premature infants and hypomorphic GR mice die neonatally due to severe lung atelectasis. Here we describe fetal lethality in GR null (GR^{-/-}) mice with 51% loss at E17.5 (p<0.05) that could be due to impaired cardiac development. Histology showed reduced heart size in GR^{-/-} fetuses from E16.5 onwards, co-incident with a peak in heart corticosterone content, which was undetectable at and before E14.5. Magnetic resonance imaging confirmed a 21.9±4.7% reduction in total ventricular volume at E17.5 (p<0.05) yet no structural abnormalities were observed. *In vivo* high frequency ultrasound analysis revealed impaired left ventricular systolic and diastolic function in E17.5 GR^{-/-} fetuses. E/A wave ratio was elevated in E17.5 GR^{-/-} fetuses (WT, 0.41±0.03 vs KO 0.28±0.04). TEI-Doppler index was increased in E17.5 GR^{-/-} fetuses (WT, 0.36±0.02 vs KO, 0.54±0.03, p<0.001), with an intermediate phenotype in heterozygous mice (0.45±0.02; p<0.01 vs WT). Impaired cardiac function was accompanied by oedema in GR^{-/-} fetuses (wet weight WT, 0.85±0.03 vs KO, 0.86±0.01, dry weight WT, 0.12±0.004 vs KO, 0.1±0.002, p<0.01; fetal Na/K ratio WT, 0.9±0.02 vs KO, 1.03±0.01, p<0.001), indicative of heart failure. The observed phenotype was not due to deficiency in cardiac adrenalin content, which was normal at E17.5, nor were there any compensatory alterations in cardiac mineralocorticoid receptor mRNA levels. Cardiomyocytes of E17.5 GR^{-/-} hearts displayed multiple cellular defects as shown by electron microscopy, including underdeveloped myofibrils and dense nuclei. RNA and protein analysis showed normal cardiac gene expression in GR^{-/-} fetuses at E14.5 (prior to the glucocorticoid increase), but a lack of the normal maturational changes at E17.5, both in mRNA and protein levels (eg increased α -myosin heavy chain, atrial natriuretic peptide, increased PGC1 α , decreased UCP2). Glucocorticoid treatment of primary murine fetal cardiomyocytes *in vitro* confirmed that glucocorticoids regulate these genes in a dose dependent manner.

These data suggest that glucocorticoid action in late gestation heart is essential for its biochemical and functional maturation and lack of the normal maturational effects of glucocorticoids leads to development of congestive heart failure in utero.

Sources of Research Support: BHF scholarship FS/08/065 awarded to EARZ.

Disclosures: BW: Investigator, Wyeth Pharmaceuticals; Ad Hoc Consultant, Boehringer Ingelheim; Astra Zeneca. Nothing to Disclose: EAR-Z, AT, CM, DGB, CJK, ZM, DS, SB, JR, EO, LF, MCH, KEC

Pub # P3-2

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Actions of Adrenal Steroid Receptors & Other Nuclear Receptors (1:30 PM-3:30 PM)

Title Cardiomyocyte-Specific Deletion of the Mouse Glucocorticoid Receptor Results in Profound Cardiovascular Disease and Morbidity

Author String R Ren, MC Boyle, JA Cidlowski
National Institute of Environmental Health Sciences, Research Triangle Park, NC; National Institute of Environmental Health Sciences, Research Triangle Park, NC

Body Stress is well known to impact cardiac function and glucocorticoids are a major mediator of stress in mammals. Corticosteroids have been suggested to be involved in the development of cardiac hypertrophy that is a major cause of congestive heart failure in both man and mice, however the mechanisms underlying this disease process are not clear. To explore the role of glucocorticoid signaling in the regulation of cardiac function in vivo, we developed mice with a cardiomyocyte specific deletion of glucocorticoid receptor (GR) by crossing α -myosin heavy chain Cre mice with GR floxed mice. The hearts of these adult homozygous mice have decreased expression of GR at both mRNA and protein levels. Unexpectedly our studies show that this GR deficiency in the heart is associated with increased morbidity in both heterozygous and homozygous mice beginning at approximately 6 months of age. In vivo cardiac function was analyzed by echocardiography and our data reveal that the conditional GR knockout mice have hypertrophic cardiomyopathy with ventricular dilation as early as one month of age. This condition progresses with increasing age. In comparison to their age-matched controls, mice lacking GR in the heart displayed a significant left ventricular dysfunction with increased end-diastolic volume, reduced fraction shortening, ejection fraction and stroke volume. Hearts from conditional GR-deletion mice and controls were further examined grossly and histologically using hematoxylin and eosin and Masson's trichrome staining. These results confirm that conditional GR deficient mice have substantially enlarged hearts not due to valvulopathy, infarction or increased myocardial fibrosis. Thus, the presence of GR is necessary for normal functioning of ventricular cardiomyocytes. Together, these data indicate an essential role for the glucocorticoid receptor in regulating cardiac function and implicate the glucocorticoid signaling pathway as a potential therapeutic target in cardiac hypertrophy and congestive heart failure.

Nothing to Disclose: RR, MCB, JAC

Pub # P3-3

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Actions of Adrenal Steroid Receptors & Other Nuclear Receptors (1:30 PM-3:30 PM)

Title Glucocorticoid Response Element (GRE) Site on the Corticotropin-Releasing Hormone (CRH) Promoter Is Important for Mediating Alcohol-Induced Changes in Promoter Activity

Author String MM Przybycien-Szymanska, TR Pak
Loyola University Medical Center, Maywood, IL; Loyola University Medical Center, Maywood, IL

Body Alcohol (EtOH) abuse during adolescence can lead to permanent changes in brain function that often manifest in adulthood as psychological disorders, such as depression and anxiety. We have previously shown that binge EtOH exposure in peri-pubertal male rats increased corticotropin-releasing hormone (CRH) mRNA in the paraventricular nucleus of the hypothalamus (PVN), a brain region responsible for coordinating stress and anxiety responses. In this study we aimed to identify the molecular mechanisms involved in mediating these effects by examining the direct effects of EtOH on CRH promoter activity in a neuronal cell line derived from the PVN (IVB). In addition, we investigated the potential interactions of EtOH and glucocorticoids on the CRH promoter by concomitantly treating cells with EtOH and the glucocorticoid receptor (GR) antagonist RU486 (100nM) and by sequentially deleting GR binding sites within glucocorticoid response element on the CRH promoter. Cells were seeded at a density of 20,000 cells/well in media containing 10% FBS and transiently transfected with a firefly luciferase reporter construct containing 2.5 kb of the rat wild type (WT) or mutated CRH promoter. Cells containing WT CRH promoter were treated 24 hours after transfection with various concentrations of EtOH (12.5 mM, 25 mM, 50 mM and 100 mM) for 2.0 h or with 100 mM EtOH for either 0.5 h, 1.0 h, 2.0 h or 4.0 h. Cells with the promoter constructs lacking specific GR binding sites were treated 24 hours after transfection with 12.5 mM concentration of EtOH for 2 h. In addition, we used a MTT toxicology assay to measure possible cell death after 100mM EtOH treatment. Our results showed that 100 mM EtOH treatment induced a biphasic response in CRH promoter activity. EtOH exposure for 0.5 and 1.0 h significantly decreased promoter activity compared to vehicle treated controls, whereas promoter activity was significantly increased after 2.0 h of EtOH exposure. After 4.0 h, the promoter activity returned back to baseline levels. Treatment with RU486, or deletion of the GR binding sites, abolished the EtOH-induced increase in the promoter activity. Overall, our data suggest that alcohol exposure directly regulates CRH promoter activity by interfering with the normal feedback mechanisms of glucocorticoids mediated by GR signaling at the CRH promoter.

Sources of Research Support: T32 AA013527 to MMS and NIH R21AA018398 to TRP.

Nothing to Disclose: MMP-S, TRP

Pub # P3-4

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Actions of Adrenal Steroid Receptors & Other Nuclear Receptors (1:30 PM-3:30 PM)

Title Glucocorticoid Receptors within Lipid Rafts Activate a MAPK-Dependent, Nongenomic Signaling Pathway To Limit Gap Junction Intercellular Communication and Proliferation in Neural Stem Cells

Author String R Samarasinghe, M Lewis, D Volonte, F Galbiati, DB DeFranco
University of Pittsburgh, Pittsburgh, PA; University of Pittsburgh School of Medicine, Pittsburgh, PA

Body Glucocorticoids (GCs) are used to treat pregnant women at risk for preterm delivery; however, prenatal exposure to GCs may trigger adverse neurological side effects mediated in part by reduced neural stem cell (NSC) proliferation. While many established cell cycle regulators impact NSC proliferation, other signaling molecules, such as the gap junction protein connexin 43 (Cx43), appear to also influence proliferation although its precise role and mechanism of regulation remain unresolved. Gap junction intercellular communication (GJIC) is influenced by GCs in some cells, but such hormone effects and resulting functional consequences have not been examined in coupled stem cells. We found that both continuous and transient dexamethasone (DEX) exposure of embryonic (E14) mouse neurosphere cultures limits proliferation of coupled NSCs, which is manifested by both a reduction in S phase progression and enhanced cell cycle exit. / short (i.e. 60 minute) DEX treatment also reduced GJIC as measured by live cell fluorescence recovery after photobleaching (FRAP). GC effects on GJIC in NSCs are transcription-independent and mediated through plasma membrane glucocorticoid receptors GRs (i.e. observed with BSA-conjugated Dex and blocked by RU486). This nongenomic pathway appears to operate through lipid-raft associated GRs through a site-specific, MAPK-dependent phosphorylation of Cx43, which is linked to GR via caveolin-1 and c-Src. As transient pharmacologic inhibition of GJIC triggers reduced S phase progression but not enhanced cell cycle exit, the nongenomic GR signaling pathway may operate via distinct downstream effectors to alter the proliferative capacity of NSCs.

Sources of Research Support: NIH Grant R01 DK078394 awarded to DBD and NIH Training Grant Fellowship T32 GM08424 awarded to RS.

Nothing to Disclose: RS, ML, DV, FG, DBD

Pub # P3-5

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Actions of Adrenal Steroid Receptors & Other Nuclear Receptors (1:30 PM-3:30 PM)

Title Glucocorticoid-Independent Repression of TNF- α -Stimulated IL-6 Expression by the Glucocorticoid Receptor: A Potential Mechanism for Protection Against an Excessive Inflammatory Response

Author String N Verhoog, C Avenant, A Kotitschke, JP Hapgood
University of Cape Town, Cape Town, South Africa

Body Tumour necrosis factor-alpha (TNF-alpha) is a potent inducer of interleukin-6 (IL-6), while ligand-activated glucocorticoid receptor (GR) is able to significantly antagonise TNF-alpha -induced IL-6 expression. TNF-alpha-signalling and IL-6 cytokine levels play a crucial role in cervical immunity and the host response to infections. We examined the role of liganded and unliganded GR in IL-6 gene regulation in response to TNF-alpha in an immortalised human endocervical epithelial cell line (End1/E6E7). In the absence of glucocorticoids, both decreasing GR protein levels by an siRNA strategy, as well as incubation with the GR antagonist RU486, resulted in potentiation of TNFalpha-induced IL-6 mRNA levels, suggesting a role for the unliganded GR in reduction of TNF-alpha-induced IL-6 expression. TNFa was shown to induce phosphorylation of the unliganded endogenous GR at Ser-226 but not Ser-211, unlike dexamethasone (DEX), which induced hyperphosphorylation at both serine residues. The GR was shown to partially translocate to the nucleus in response to TNF-alpha. Furthermore, TNF-alpha induced recruitment of the unliganded GR to the IL-6 promoter to a similar extent as DEX. Taken together these results show that unliganded GR undergoes activation and nuclear translocation and actively attenuates IL-6 gene expression, in response to TNF-alpha. In addition, TNF-alpha also induced the recruitment of GRIP-1 to the IL-6 promoter and overexpression studies are consistent with a function for GRIP-1 as a co-repressor in this context. These findings suggest the presence of a novel auto-regulatory mechanism that may prevent overproduction of IL-6 in the endocervix, possibly protecting against negative effects of excessive inflammation.

Nothing to Disclose: NV, CA, AK, JPH

Pub # P3-6

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Actions of Adrenal Steroid Receptors & Other Nuclear Receptors (1:30 PM-3:30 PM)

Title Functional Consequences of a Naturally Occurring Osmolyte-Induced Folding of Intrinsically Disordered AF Domain of the Glucocorticoid Receptor

Author String SH Khan, R Kumar
The Commonwealth Medical College, Scranton, PA

Body Intrinsically disordered (ID) regions are frequently found in the activation domains of many transcription factors including nuclear hormone receptors. It is generally believed that ID nature of these activation domains promote molecular recognition by creating large interaction surfaces suitable for binding with their specific protein binding partners, which is a critical component of gene regulation by transcription factors. It has been hypothesized that conditional folding of these activation domains may be a prerequisite for their efficient interaction with specific coregulatory proteins, and subsequent transcriptional activity leading to the regulation of target gene(s). Glucocorticoid receptor (GR) mediates the biological effects of glucocorticoids at the level of gene regulation. To initiate transcription of target gene(s), GR interacts with its response element DNA, and/or with various coregulatory proteins. However, specific interaction surfaces of GR with its coregulators are not well understood. Consequently, precisely how transcription is regulated by GR is largely unknown. This is due, in part, to the lack of structural information about GR's major transactivation function region, AF1 located in the N-terminal domain. The major obstacle in determining the structure of AF1 has been due to its ID conformation. In this study, we tested whether a naturally occurring osmolyte, trehalose can promote functionally ordered conformation in AF1. Our data show that in the presence of trehalose, AF1 acquires secondary/tertiary structure such that its interaction with steroid receptor coactivator-1 (SRC-1), a critical coregulator of glucocorticoid receptor's activity, is greatly enhanced. Our results may provide a mechanism for cell-type specificity of the effects of GR in gene regulation.

Sources of Research Support: NIH RO1 Grant: DK058829.

Nothing to Disclose: SHK, RK

Pub # P3-7

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Actions of Adrenal Steroid Receptors & Other Nuclear Receptors (1:30 PM-3:30 PM)

Title Structure and Functions of the N-Terminal Activation Function (AF1) Domain of the Glucocorticoid Receptor

Author String SH Khan, R Kumar
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Body The precise mechanism by which glucocorticoid receptor (GR) regulates the transcription of its target genes is largely unknown. This is, in part, due to the lack of structural and functional information about GR's activation function (AF1) region located within the N-terminal domain. Like the N-terminal activation domain of many steroid receptors, the GR AF1 exists in an intrinsically disordered (ID) conformation or an ensemble of conformers that collectively appears to be unstructured. The GR AF1 is known to recruit several coregulatory proteins, including those from the basal transcriptional machinery, e.g. TATA box binding protein (TBP). It is interesting that the ID GR AF1 directly interacts with the TBP, the critical protein that forms the basis for the multiprotein transcription initiation complex. However, the precise mechanism of this process is unknown. We have earlier shown that conditional folding of the GR AF1 is the key for its interactions with critical coactivator proteins, including steroid receptor coactivator-1 (SRC-1). We hypothesize that binding of TBP to AF1 results in the structural rearrangement of the ID AF1 domain such that its surfaces become easily accessible for interaction with other coactivators. To test this hypothesis, we determined whether TBP binding-induced structure formation in the GR AF1 facilitates its interaction with SRC-1, a critical coactivator which is important for GR-mediated transcriptional activity. Our data show that stoichiometric binding of TBP induces significantly higher helical content at the expense of random coil configuration in the GR AF1. Further, we found that this induced AF1 conformation facilitates its interaction with SRC-1, and subsequent AF1-mediated transcriptional activity. Our results provide a potential mechanism through which GR AF1 may regulate the expression of the GR-target genes, information essential to understand how specific signals are passed from the receptor to target genes.

Sources of Research Support: NIH RO1 Grant: DK058829.

Nothing to Disclose: SHK, RK

Pub # P3-8

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Actions of Adrenal Steroid Receptors & Other Nuclear Receptors (1:30 PM-3:30 PM)

Title Differential Requirements of GR Regulatory Surfaces and Coregulators in Glucocorticoid-Regulated Transcription of Different Target Genes

Author String R Chodankar, B Schiller, L Watson, K Yamamoto, S Micahel
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Body Hormone-activated glucocorticoid receptor (GR) regulates transcription of several hundred genes in any given cell type. But the extent, mechanism, and nature (i.e. activation or repression) of regulation varies widely among the target genes. Factors which determine the outcome include the DNA sequence of the GR binding site, the resulting conformation of GR, the activation surfaces of GR required, and the specific coregulators recruited and required at each target gene. The goal of our study is to conduct an integrated analysis of all of these parameters and thereby determine how they combine to specify different mechanisms of regulation for different GR target genes. We used a series of U2OS osteosarcoma cell lines that stably overexpress a wild-type GR- α or GR with mutations in activation domains AF1, AF2, or the dimerization interface of the DBD (GR-dim). A global analysis using Illumina Beadchip gene expression microarrays defined subsets of GR target genes whose induction by dexamethasone depends on the various functional surfaces of GR. We are also depleting specific coregulators by RNA interference to analyze whether coregulator requirements correlate with requirements for GR surfaces. Our initial results indicate that endogenous GR target genes were differentially responsive to AF1, AF2 and Dim mutations. Interestingly, we found that the Dim mutation causes loss of hormonal regulation of some GR target genes, but also causes regulation of new target genes that are not regulated by wild type GR. We further used Chip-seq to analyze differences in DNA binding by wt GR and GR-dim. In some cases the differences in gene regulation were due to differences in GR binding to the target genes, while in other cases target genes were bound similarly by wt GR and GR-dim, but the regulation in response to hormone was different. In addition to differential GR binding at some target genes, we are currently exploring whether differential coregulator recruitment by GR wt and GR-dim contribute to their differential regulation of specific target genes.

Nothing to Disclose: RC, BS, LW, KY, SM

Pub # P3-9

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Actions of Adrenal Steroid Receptors & Other Nuclear Receptors (1:30 PM-3:30 PM)

Title Glucocorticoid-Mediated Changes in Adipose Triacylglycerol Metabolism Are Exacerbated in the Absence of a Functional GR Dimerization Domain

Author String DJ Roohk, M Hellerstein, CA Harris
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Body Glucocorticoids (GCs) are effective treatments for a wide variety of inflammatory diseases, but their use is limited by adverse metabolic effects including osteoporosis, hypertension, hyperlipidemia, obesity and insulin resistance. GCs act via the GC receptor (GR), a nuclear hormone receptor. GR has multiple effector arms including dimerization mediated transactivation of target genes via DNA binding and transcriptional repression mediated by protein-protein interactions. Because the major repressed transcription factors AP-1 and NF- κ B are critical for the immune response, a model has emerged in which repression mediates the anti-inflammatory properties of GCs and the adverse effects are mediated by transactivation(1). Much attention has been focused on creating selective GR modulators with improved side effect profiles (2). The GRdim mouse has a mutation in the dimerization domain and therefore displays attenuated transactivation with intact repression(3). In order to understand the role of GR dimerization dependent targets in skin and adipose tissue physiology, we have examined skin collagen synthesis and adipose TG synthesis in vivo via stable isotope studies in response to the potent GC dexamethasone (DEX) in WT and GRdim mice. Mice were injected with 10 mg/kg DEX every other day for 7 days. During the treatment period mice were given an i.p. bolus of D2O (3.5% v/w in saline) and then given 8% D2O in their drinking water. Mass isotope distribution analyses were performed on collagen extracted from skin and TG extracted from adipose tissue. WT and GRdim mice both demonstrated suppression of skin collagen synthesis induced by DEX. Both WT and GRdim mice showed DEX dependent increases in TG synthesis and DNL in the inguinal and epididymal fat depots. GRdim mice showed an exaggerated DNL response to DEX in both depots. Although the GRdim mutation has classically been described as a loss of function hypomorph, more recent examinations have uncovered some gain of function properties of the mutation resulting in gene activation by GCs that do not occur with WT GR. It is therefore possible that the enhanced TG synthesis seen in GRdim mice is due to loss of activation of a GR dimerization dependent target that is normally a negative regulator of TG synthesis or due to [ldquo]rogue[rdquo] activation by GRdim of a positive regulator of TG synthesis. Gene expression studies are underway to determine the mechanism for the altered lipid metabolism seen in GRdim mice.

(1) Sch[au]m]cke et al Curr Opin Investig Drugs. 2005;6(5):503-7.

(2) De Bosscher et al Curr Opin Pharmacol 2010; 10(4):497-504

(3) Reichardt et al Cell 1998;93(4):531-41

Sources of Research Support: NIDDK Grant 5K08DK081680 to CAH.

Nothing to Disclose: DJR, MH, CAH

Pub # P3-10

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Actions of Adrenal Steroid Receptors & Other Nuclear Receptors (1:30 PM-3:30 PM)

Title Sustained Prednisolone Treatment Leads to Excessive Dyslipidemia in Mice Carrying a Dimerization-Defective Glucocorticoid Receptor

Author String AJ Laskewitz, A Rauch, A Grefhorst, F Kuipers, JP Tuckerman, BK Groen
University Medical Center Groningen, Groningen, Netherlands; Leibniz Institute for Age Research - Fritz Lipmann Institute, Jena, Germany; Erasmus Medical Center, Rotterdam, Netherlands

Body
Background: Synthetic glucocorticoids such as prednisolone are potent anti-inflammatory drugs. Their use, however, is limited by the severe side effects they may induce. Many of the side effects resemble features of the metabolic syndrome, such as weight gain, insulin resistance and dyslipidemia. Glucocorticoids act via the glucocorticoid receptor (GR), which shows activity both as dimer or monomer, leading to metabolic regulation via either transcriptional activity or cytosolic protein-protein interactions. To segregate these effects, a mouse model has been developed with a mutation in the dimerization part of the GR, in this GRdim mouse only monomeric GR is active. We aimed to elucidate the effects of chronic prednisolone treatment on metabolic profiles in GRdim and wild type mice.
Methods: WT and GRdim mice received sustained treatment for 6 days, by implantation of a prednisolone pellet. Before and during the treatment, plasma glucose levels and lipoprotein profiles were determined. After 6 days, livers were collected and analyzed for lipid levels and gene-expression pattern.
Results: Six day prednisolone treatment did not influence fasting glucose levels in GRdim mice, but strongly increased plasma triglyceride and cholesterol levels. This was already apparent after one day of treatment. Prednisolone treatment resulted in a dramatic 9-12 fold increase of triglycerides and free cholesterol in the VLDL fraction of prednisolone treated GRdim mice. Surprisingly, hepatic cholesterol and phospholipid levels did not change, while hepatic triglyceride levels were reduced in prednisolone treated GRdim mice. The unusual lipid profile was not caused by cholestasis since plasma bile acid concentration did not increase. Hepatic gene expression levels showed no major differences in genes involved in cholesterol conversion and lipoprotein metabolism as well as in bile acid secretion and production. Interestingly, gene expression levels of lipoprotein lipase (*lpl*) were 23 times induced.
Conclusion: Prednisolone treatment in GRdim mice leads to formation of an atypical VLDL particle containing high levels of triglyceride, phospholipids and free cholesterol. These results indicate an important role of monomeric GR in regulation of lipid metabolism and this may contribute to unravelling the mechanisms underlying glucocorticoid-induced side effects.

Sources of Research Support: This work was performed within the framework of the Dutch Top Institute Pharma T1-106.

Nothing to Disclose: AJL, AR, AG, FK, JPT, BKG

Pub # P3-11

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Actions of Adrenal Steroid Receptors & Other Nuclear Receptors (1:30 PM-3:30 PM)

Title Comparison of the Glucocorticoid and Mineralcorticoid Receptor Transcriptomes in Primary Human Adipose Tissue

Author String CO Brock, JE Park, PA Volden, S Kashyap, SD Conzen
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Body In addition to its classical role as an energy storage depot, adipose tissue has more recently been recognized as an independent endocrine organ that can influence several metabolic diseases. Both glucocorticoids and aldosterone appear to alter adipose tissue biology, affecting adipogenesis, secretion of inflammatory adipokines, and insulin resistance. Thus the glucocorticoid receptor (GR) and the mineralocorticoid receptor (MR) which are the cognate receptors for cortisol and aldosterone respectively, may target genes with important roles in diabetes, obesity, hypertension and the metabolic syndrome. To date, there has been a paucity of data about the genome-wide transcriptional targets of either the GR or MR in primary human fat. We sought to determine whether the MR and GR share common targets or gene networks in primary human adipocytes that may underlie the connection between cortisol and aldosterone with regard to their effects in fat and metabolic disease. Thus far, we have confirmed MR and GR mRNA and protein expression in primary human adipocytes isolated from subcutaneous adipose tissue using RT-PCR and Western analysis, respectively. We have also immunoprecipitated the GR and performed massively parallel sequencing of the GR-bound chromatin (ChIP-seq). We are also performing the ChIP-seq experiment with the immunoprecipitated MR. Genome-wide chromatin regions identified as interacting with both the GR and MR in a ligand-dependent manner will be mapped and compared to gene expression profiles following GR and MR activation. Based on our preliminary data, the MR and GR appear to bind to predominantly distinct regions of the human genome although they do regulate a subset of overlapping genes including SGK1. By identifying the distinct and overlapping GR and MR transcriptome we will begin to understand the role of these nuclear receptors in adipose tissue biology and metabolic disease, and determine whether the receptors, or the genes they regulate, may be useful therapeutic targets.

Sources of Research Support: HHMI Medical Student Fellowship to COB, Avon Foundation Grant and NIH Grant R01 to SDC.

Nothing to Disclose: COB, JEP, PAV, SK, SDC

Pub # P3-12

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Actions of Adrenal Steroid Receptors & Other Nuclear Receptors (1:30 PM-3:30 PM)

Title The Mineralocorticoid Receptor: Identification of Ligand-Specific Interacting Proteins Using High-Throughput T7 Phage Display

Author String J Yang, CD Clyne, R Safi, C-y Chang, PJ Fuller, DP McDonnell, MJ Young
Prince Henry's Institute, Clayton, Australia; Duke University Medical Center, Durham, NC

Body The mineralocorticoid receptor (MR) is unique in its ability to bind both glucocorticoids (cortisol) and mineralocorticoids (aldosterone, deoxycorticosterone). Although all three physiologic ligands can act as agonists of the MR in the kidney, cortisol can act as an antagonist in other tissues such as the heart. Pathological activation of the cardiac MR causes fibrosis and heart failure. MR antagonists have been shown to reduce morbidity and mortality from heart failure, but their clinical use is limited by the renal side effect of hyperkalemia. To develop a selective MR modulator, the mechanisms for the ligand- and tissue-specific actions of the MR need to be defined. The key may lie with the structurally and functionally diverse coregulator proteins which are critical for nuclear receptor mediated gene expression. We hypothesize that different ligands induce distinct MR conformations to allow differential coregulator recruitment and ligand-specific gene expression. This is supported by our previous work which identified ligand-selective MR-interacting 19mer peptides using M13 phage display [1]. To isolate more biologically relevant peptides, we have screened a T7 phage display cDNA expression library derived from the human heart to identify proteins that interact with the full-length MR in the presence of aldosterone, cortisol or deoxycorticosterone (DOC). The isolated peptides, ranging in length from 50 to 200 amino acids, were assessed for a functional interaction with the MR in transactivation interference assays in CV-1 cells. We identified two peptides (F20b and A44b) which strongly inhibited MR transactivation in the presence of all three ligands. One other peptide (A38) demonstrated ligand-selectivity with partial inhibition of MR transactivation in the presence of aldosterone and DOC but not cortisol. Interestingly none contained the conserved NR-binding motifs found in well characterized coregulators. These peptides are currently being assessed for their ability to directly interact with the MR and, as full-length proteins, to coregulate MR-mediated transactivation. They represent novel MR-interacting proteins which expand our knowledge of MR signaling and contribute to the ultimate cohort of ligand- and tissue-specific MR binding proteins that may be targeted in the rational design of a selective cardiac-specific MR modulator.

(1) Yang, J., et al., Identification of Ligand-Selective Peptide Antagonists of the Mineralocorticoid Receptor Using Phage Display. *Mol Endocrinol*, 2011. 25(1): 32-43.

Sources of Research Support: National Health and Medical Research Council of Australia Grant 494835 awarded to JY.

Nothing to Disclose: JY, CDC, RS, C-YC, PJF, DPM, MJY

Pub # P3-13

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Actions of Adrenal Steroid Receptors & Other Nuclear Receptors (1:30 PM-3:30 PM)

Title Severe Autosomal Recessive Pseudohypoaldosteronism Type 1 Caused by Mutations in the Mineralocorticoid Receptor Gene

Author String E-L Hubert, R Teissier, FL Fernandes-Rosa, M Fay, M-E Rafestain-Oblin, X Jeunemaitre, C Metz, B Escoubet M-C Zennaro
Inserm, Paris, France; University Paris Descartes, Paris, France; Centre Hospitalo-Universitaire de Brest, Brest, France; Inserm, Paris, France; University Paris Diderot - Paris 7, Paris, France; Assistance Publique - H[ocirc]pitaux de Paris, Paris, France; Assistance Publique - H[ocirc]pitaux de Paris, Paris, France

Body Renal pseudohypoaldosteronism type 1 (PHA1) is a rare autosomal dominant form of mineralocorticoid resistance characterized by salt wasting and failure to thrive in infancy. We report a unique pedigree, in which the index case was diagnosed with renal PHA1. Diagnosis was confirmed by genetic testing of the NR3C2 gene, coding for the mineralocorticoid receptor (MR), which showed a frameshift mutation in exon 2 (c.497-498delCT) leading to replacement of Ser166 by a stop codon (p.Ser166X). One sibling presented with an extremely severe phenotype: at 2 years of age the child presents growth delay (-2 SDS) and moderate mental retardation and still requires 31 mEq[frasl]kg[frasl]d of sodium supplementation. Clinical, biochemical and genetic investigation of the family discovered independent segregation of two NR3C2 mutations, p.Ser166X from the affected father, and a second nonsense mutation in exon 6 (c.2418G>A[frasl]p.Trp806X), from the asymptomatic mother. The child presenting with severe PHA1 was compound heterozygous for both mutations.

While MR transcripts from both alleles were expressed in patient's lymphocytes, only MR_{806X} was detected as a ~90 kDa protein, suggesting that the mutation p.Ser166X leads to protein degradation. Truncation of the MR_{806X} at the very beginning of the ligand binding domain completely abolished hormone binding. Surprisingly, MR_{806X} was constitutively active in RCSV3 and COS-7 cells, possessing ~25% and ~45% respectively of transcriptional activity observed with the MR_{WT} at 10⁻⁸ M aldosterone. MR_{806X} showed increased nuclear localization as compared to MR_{WT}, independently of hormone addition, explaining in part the mechanism whereby MR_{806X} had acquired its partial ligand-independent transcriptional activity. However, MR_{806X} had completely lost the capacity to recruit SRC-2 both in the absence or presence of aldosterone in RCSV3, HEK293T, and COS-7 cells, indicating that the repertoire of transcriptional coactivators recruited to the MR_{806X} is decreased. This exceptional case demonstrates that a minimal residual activity of the MR is compatible with life. It also suggests that rare hypomorphic NR3C2 alleles may be more common in the general population than expected from the prevalence of detected PHA1 cases. Heterozygous carriers may have a slightly up-regulated renin-angiotensin-aldosterone axis which may modulate their vulnerability for hypertension and cardiovascular disease.

Sources of Research Support: INSERM and by a national clinical research program funded by the Ministry of Health (PHRC P070139, Cardiovascular Evaluation of Adult PHA 1 Patients, PHACARV, Assistance Publique - H[ocirc]pitaux de Paris, NCT00646828).

Nothing to Disclose: E-LH, RT, FLF-R, MF, M-ER-O, XJ, CM, BE, M-CZ

Pub # P3-14

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Actions of Adrenal Steroid Receptors & Other Nuclear Receptors (1:30 PM-3:30 PM)

Title Protein Inhibitor of Activated STATs as Novel Co-Regulator of the Vitamin D Signal Pathway

Author String S Jena, WP Lee, PD Thompson
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Body Protein inhibitors of activated STAT (PIAS) represent a family of proteins initially identified as inhibitors of STAT (signal transducer and activator of transcription) signalling but which have emerged over the past decade as also having potent modulatory effects upon the activities of a number of steroid hormone receptors such as those for androgen and estrogen. In contrast, relatively little is known as to what role PIAS proteins may play within those signal pathways initiated by nutrient-derived molecules such as vitamin D. The endocrine responses to vitamin D are mediated through a heterodimeric complex consisting of the nuclear vitamin D receptor (VDR) and retinoid X receptor (RXR) that forms temporal associations with various co-regulatory proteins to orchestrate an appropriate transcriptional network of target genes. A possible involvement of PIAS proteins within this pathway was indirectly suggested by an earlier report that demonstrated a functional interaction between STAT 1 and VDR in immune cells (1).
The present study investigated the potential for different members of the PIAS family to impact upon the vitamin D response. We report that among the different PIAS isoforms evaluated, co-expression of PIAS4 in HEK-293 and MCF-7 breast cancer cells resulted in a striking and robust inhibition of CYP3A4 and CYP24 promoter transactivation by vitamin D, when assessed through use of both luciferase-based reporter assays and real time expression analysis of the endogenous CYP24 gene. We demonstrate that such repression of the vitamin D response is dependent upon the SUMO-ligase function of PIAS4, with VDR and PIAS4 exhibiting an association as revealed through co-immunoprecipitation assays. Finally, we report that PIAS4 functions to catalyze the conjugation of VDR with SUMO, a modification that is reversed upon binding of ligand by the receptor. In total, our results signify PIAS4 and the post-translational process of SUMOylation as important modulators of VDR function, a finding that may provide important insights as how vitamin D can achieve its pleiotropic effects.

(1) Vidal M et al., Mol Cell Biol 2002; 22:2777

Sources of Research Support: Department of employment and learning, Northern Ireland.

Nothing to Disclose: SJ, WPL, PDT

Pub # P3-15

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Actions of Adrenal Steroid Receptors & Other Nuclear Receptors (1:30 PM-3:30 PM)

Title Microarray Analysis of VDR^{-/-} Keratinocyte Stem Cells

Author String HF Luderer, MB Demay
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Body Ligand independent functions of the Vitamin D Receptor (VDR) unique to the skin are required for normal hair cycling in humans and mice. Mice lacking the VDR develop alopecia totalis accompanied by lipid laden dermal cysts. Functional deficits in the self-renewal and lineage commitment of Keratinocyte Stem Cells (KSCs), which give rise to all the lineages of the pilosebaceous unit, were observed in *vdr*^{-/-} mice. Elucidating the functional role of the VDR in the KSC has translational applications in identifying molecular targets for treatment of disorders of the skin and its appendages.

To identify pathways altered in the absence of the VDR, microarray analysis of KSCs isolated from wild type and *vdr*^{-/-} mice at postnatal day 28 was performed using Affymetrix Mouse Gene 1.0 ST arrays. More than 150 differentially regulated genes were identified in *vdr*^{-/-} KSC compared to wild type using dChip comparative analysis. Two differentially regulated genes important in post natal hair cycling were identified: the nuclear receptor corepressor hairless (HR) and B lymphocyte maturation factor 1 (Blimp-1). In the absence of the VDR, a 2.5-fold increase in Hr gene expression and a 2-fold decrease in Blimp-1 gene expression was observed. Gene ontology analysis using Metacore software also detected significant alteration in the cholesterol biosynthesis and lipid metabolism pathways, consistent with the role of the VDR in regulating lipid metabolism in the epidermis.

Sources of Research Support: NIAMS F32 AR056933-01A1; NIDDK T32 DK007028.

Nothing to Disclose: HFL, MBD

Pub # P3-16

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Actions of Adrenal Steroid Receptors & Other Nuclear Receptors (1:30 PM-3:30 PM)

Title Changes in Nuclear Receptor and Coregulator Gene Expression during Osteoblastic Differentiation

Author String MM Roforth, S Khosla, DG Monroe
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Body A complex network of transcription factors contributes to the establishment and maintenance of the osteoblastic phenotype. Although relatively few transcription factors, such as Runx2 and osterix, are essential to the process of osteoblastic differentiation, others serve the purpose of fine-tuning in response to various environmental and hormonal cues. In this study we report the expression patterns of the 49 members of the nuclear hormone superfamily and 30 well-characterized coregulators during osteoblastic differentiation using a primary mouse calvarial cell model. Cells were treated at confluence with standard osteoblastic differentiation media (ascorbic acid, β -glycerophosphate) and samples collected at various timepoints. The transcriptional effects of early osteoblastic differentiation (0-24 hr) and late differentiation (7-16 days) were assessed using QPCR analysis followed by cluster analysis of present (Ct<33) and statistically significant ($p<0.05$) genes derived from the dataset. Transcriptional differences were observed 2 hr following the onset of osteoblastic differentiation, including select nuclear receptors (NGFIB, NOR1, RAR α , PPAR γ , PPAR[delta]), members of the AP1 family (c-jun, Fra1, Oasis) and other factors with known involvement in osteoblast biology (Hes1, Twist1/2, Msx2, NFATc1, Dlx2). A group of nuclear receptors with peak induction at 24 hr following differentiation (FXR α , LXR α , RAR β , GCNF, TR β , ERR γ , ER α and ER β) was observed along with upregulation of the classic bone marker genes alkaline phosphatase (AP) and osteocalcin (OC). Interestingly, SRC1 and ER α tightly clustered, confirming the close association of these two factors in many systems. At longer periods of osteoblastic differentiation (7-16 days), the majority of genes were downregulated (~75%). Notable exceptions include LXR α , ER α , ROR γ , VDR, PR and TR β , suggesting these factors may be important in late osteoblastic differentiation. The bone marker genes Runx2, osterix, AP, OC, Col1A1, and Dlx5/6 were upregulated, confirming late-stage osteoblastic differentiation. Collectively, these data demonstrate that large-scale changes in the nuclear receptor superfamily and select coregulators occur throughout osteoblastic differentiation, possibly leading to altered sensitivity to various hormonal cues. Whether these changes are prerequisite for osteoblastic differentiation or a consequence of the induction of differentiation remains a major unanswered question.

Nothing to Disclose: MMR, SK, DGM

Pub # P3-17

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Actions of Adrenal Steroid Receptors & Other Nuclear Receptors (1:30 PM-3:30 PM)

Title Proteasome Inhibitor, but Not Cycloheximide, Commonly Alleviates Suppression of PTHrP Expression Mediated by Several Steroid Hormones in Breast Cancer Cells

Author String T Kajitani, M Iizuka, M Tamamori-Adachi, T Okazaki
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Body PTHrP produced by bone-metastasizing cancer cells, especially breast cancers, stimulates bone resorption, leading to release of TGF-beta and IGF-1, which again enhances PTHrP secretion, thus forming a deadly vicious cycle. Along with the above TGF-beta or IGF-1, hedgehog-Gli2 and EGF pathways are known to be strong stimulators of PTHrP production. Unfortunately, there have been no known molecules significantly repressing PTHrP overproduction in this setting. Previous studies have scatteringly reported that several steroid hormones negatively regulate expression of PTHrP. Here we studied PTHrP expression in response to steroid hormones in two breast cancer cell lines, MCF-7 and T47D. Our study confirmed that steroid hormones inhibited PTHrP expression in these cells, and clear correlations between the expression of the nuclear receptors (NRs) and the steroid hormone-induced PTHrP suppression were observed. Knocking-down by siRNA for NRs in MCF-7 cells revealed that vitamin D receptor, estrogen receptor (ER)alpha, progesterone receptor, and glucocorticoid receptor, were required for repression of PTHrP expression by the corresponding ligands. A notable exception was the androgen receptor (AR), which was dispensable for suppression of PTHrP expression in dihydrotestosterone (DHT)-treated cells. Furthermore, experiments with siRNA for ERalpha and ER antagonist ICI182780 strongly suggested that DHT-induced suppression of PTHrP expression appeared to be mediated by ERalpha, but not by AR. By using cycloheximide, we found such inhibition was brought about through a transcriptional process. On the other hand, vitamin D3-mediated inhibition on the steady-state level of PTHrP mRNA, which was recognized in MCF-7, but not in T47D cells, was a post-transcriptional event. Chromatin immunoprecipitation assay by using MCF-7 cells identified at least two distinct far upstream regions of human PTHrP gene which recruited these NRs in common. Further, we found that proteasome inhibitor MG132 completely abolished the repressive effects of all the steroid hormones. These results suggest that a cyclic and ordered degradation of a repressive transcriptional complex (es) including respective NR is necessary for PTHrP suppression.

Nothing to Disclose: TK, MI, MT-A, TO

Pub # P3-18

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Actions of Adrenal Steroid Receptors & Other Nuclear Receptors (1:30 PM-3:30 PM)

Title Transcriptomine: A Community Web Research Resource for Regulation of Gene Expression by Nuclear Receptors and Their Ligands

Author String SA Ochsner, L Becnel-Boyd, CM Watkins, BS LaGrone, A McOwiti, D Steffen, NJ McKenna
Baylor College of Medicine, Houston, TX; Baylor College of Medicine, Houston, TX

Body Understanding signaling by nuclear receptors (NRs) requires an appreciation of their cognate ligand- and tissue-specific transcriptomes. While target gene regulation data are abundant in the field, they reside in hundreds of discrete publications in formats refractory to routine query and analysis and, accordingly, their full value to the NR signaling community has not been realized. One of the mandates of the Nuclear Receptor Signaling Atlas (NURSA) is to facilitate access of the community to existing public datasets. Pursuant to this mandate we are developing a freely-accessible community web resource, Transcriptomine, that for the first time will bring together the sum total of available expression array data points generated by the field in a single location. Transcriptomine will provide for intuitive querying of a [ldquo]super-dataset[rdquo] of approximately 3700 individual experiments, encompassing 35 nuclear receptors, 95 NR ligands, 350 unique tissues & cell lines and 44 million array data points, extracted from a total of 930 published studies. Transcriptomine is designed to accommodate a spectrum of end users ranging from the bench researcher to those with advanced bioinformatic training. Our resource affords an entirely new paradigm for leveraging gene expression data in the NR signaling field and will, when complete, empower users to assemble complex queries that span diverse molecules, tissues & cell lines, target genes, biological functions and disease associations, and that would otherwise be prohibitive in terms of time and effort. Transcriptomine will be regularly updated with gene lists from future genome-wide expression array and expression-sequencing datasets in the NR signaling field.

Sources of Research Support: National Institute of Diabetes, Digestive and Kidney Disease Nuclear Receptor Signaling Atlas Grant U19 DK62434, with supplementary support from the National Institute of Environmental Health Sciences and the National Heart, Lung and Blood Institute.

Nothing to Disclose: SAO, LB-B, CMW, BSL, AM, DS, NJM

Pub # P3-19

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Molecular & Physiological Actions of the Sex Steroid Receptors (1:30 PM-3:30 PM)

Title A Dynamic and Genome-Wide Estrogen Receptor Cistrome

Author String T Westerling, LE Tavera-Mendoza, M Brown
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Body
The function and regulation of Estrogen Receptor alpha (ER) is important for the homeostasis of several cell types and organs and the misregulation or lack of ER is involved in a number of human pathologies including breast cancer and osteoporosis. Genome-wide binding data of ER and some associated co-factors have yielded a wealth of understanding of the normal function of ER and the role of cofactors. These Cistromes ('genome-wide cis-acting targets of trans-acting factors') have illuminated the function of ER both as an ubiquitous regulatory mechanism shared for some genes in several cell types but also for cell type specific regulatory patterns. The detailed molecular mechanisms of dynamic assembly of the ER machinery on the chromatin has also been studied using only a limited number of known regulatory sites. However, efforts to combine the dynamic behavior of ER binding to chromatin and genome-wide binding analysis have not been previously undertaken.
In this study we present data on a dynamic Cistrome for ER. We determine the genome-wide binding profiles (ChIP-chip and ChIP-seq) of ER and estimate relative binding affinities on various site over a E2-stimulation time course in order to characterize binding sites with binding modalities that differs from the classical 45 minute steady cycling. In order to validate and quantify our findings we have employed the Nanostring platform and find that the cycling of ER first found on a few selected promoter is indeed a genome wide phenomenon. It is interesting to note that not all binding sites follow the classical cycling pattern suggesting the presence of hitherto uncharacterized regulatory mechanism of ER itself or transcription factor cycling in general.

Nothing to Disclose: TW, LET-M, MB

Pub # P3-20

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Molecular & Physiological Actions of the Sex Steroid Receptors (1:30 PM-3:30 PM)

Title Aromatase/ER α /Tnfrsf12a Activation in Skeletal Muscle Causes Large Scrotal Hernias in Mice

Author String H Zhao, EK Pearson, DC Brooks, T Kurita, D Berger, M Demura, FJ DeMayo, SE Bulun
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Body In the US, more than 1 in 4 men can expect to develop symptomatic inguinal hernia, and more than 600,000 inguinal hernia repair surgeries are performed annually. Despite its prevalence as a major public health issue, the biologic or genetic basis of inguinal hernia is currently unknown. Previously, male rodents treated with exogenous estrogen have observed to develop large scrotal hernias that are analogous to inguinal hernias in men. The biologically active estrogen, estradiol, is synthesized by the aromatase enzyme. The human aromatase gene is different than its mouse counterpart in that it is expressed in peripheral tissues such as skeletal muscle and fat in addition to testes and the brain. To establish a mouse model for studying the underlying mechanisms of scrotal hernia, we recently generated 2 transgenic humanized aromatase (Arom^{hum}) mouse lines, each containing a single copy of the entire human aromatase gene to mimic human physiology with respect to estrogen production. Arom^{hum} mice expressed the aromatase gene in many peripheral tissues including skeletal muscle, but circulating levels of estradiol remained similar to those in wild-type (WT) controls. Male Arom^{hum} mice exhibited a progressive loss of muscle fiber and bulging of the lower abdominal wall, first noticeable at 4 weeks, resulting in large scrotal hernia in 90-100% of mice in either line by 24 weeks, whereas none of the WT controls had this phenotype. Histology indicated accelerated degeneration of lower abdominal muscle fibers replaced by fibrosis. We found severely increased levels of estrogen receptor- α (ER α) in lower abdominal muscle compared with upper abdominal muscle of normal morphology and strength. A microarray analysis demonstrated increased expression of *Tnfrsf12a*, the receptor for TNF-related weak inducer of apoptosis, in lower abdominal muscle tissue of Arom^{hum} mice vs. WT controls. Real-time RT-PCR analysis verified this result. From 3 to 16 weeks, systemic administration of the aromatase inhibitor letrozole prevented the occurrence of hernia in all animals and prevented degenerative changes in lower abdominal muscle. We concluded that estradiol via ER α activated TNF signaling, which enhanced lower abdominal muscle degeneration giving rise to hernia. Our findings resonate with remarkable and parallel increases in inguinal hernia incidence and aromatase expression in skeletal muscle and fat tissues with advancing age in men.

Nothing to Disclose: HZ, EKP, DCB, TK, DB, MD, FJD, SEB

Pub # P3-21

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Molecular & Physiological Actions of the Sex Steroid Receptors (1:30 PM-3:30 PM)

Title Synergistic Actions of LRH-1 and ER α in Breast Cancer

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Body Liver Receptor Homolog 1 (LRH-1, NR5A2), an orphan nuclear receptor, plays important roles in embryonic development, cholesterol and bile acid homeostasis, and steroidogenesis. In intestinal cancers it promotes cell proliferation and cell cycle progression. High LRH-1 expression has been demonstrated in the epithelial compartment of both invasive ductal carcinoma and ductal carcinoma *in situ*. Its expression positively correlates with estrogen receptor α (ER α) status and aromatase activity; and LRH-1 promotes estrogen-dependent cell proliferation. By silencing or over-expressing LRH-1 in breast cancer cells, we have recently shown that LRH-1 promotes cell motility and invasiveness (1). Similar effects are observed in the non-tumorigenic mammary epithelial cell line, MCF-10A. Remodelling of the actin cytoskeleton, post-translational modification of E-cadherin and an increase in MMP9 expression are in LRH-1 over-expressing cells; and likely to contribute to increased migratory and invasive properties. The current study aimed to identify molecular mechanisms regulated by LRH-1 in breast cancer epithelial cells. Using Illumina whole genome profiling, LRH-1 regulated genes were identified by silencing or over-expressing LRH-1 in MCF-7 (ER+) cells. Analysis indicated that a many LRH-1 target genes are also direct ER α targets. Chromatin immunoprecipitation with LRH-1 antibody demonstrates LRH-1 occupancy at ER α response elements (ERE) in the promoters of ER α targets. LRH-1 binds directly to the ERE as demonstrated by electrophoretic mobility shift assay. Additionally, LRH-1 acts synergistically with ER α to activate transcription of ERE containing promoter elements and increase cell proliferation. These findings suggest that in ER+ breast cancer cells, LRH-1 can enhance of ER α mediated actions by increased transcription of target genes such as GREB-1 and increased cell proliferation. Further investigation of LRH-1 in ER+ and ER- tumour cells is required to understand its impact in breast cancer progression. As LRH-1 is an orphan nuclear receptor, it is a druggable target, raising the possibility of LRH-1 as a target for novel breast cancer therapeutics.

(1) Chand AL et al., Endocrine Related Cancer 2010; 17(4):965-75

Sources of Research Support: Victorian Breast Cancer Consortium; Victorian Cancer Agency Early Career Seed Grant.

Nothing to Disclose: ALC, CDC

Pub # P3-22

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Molecular & Physiological Actions of the Sex Steroid Receptors (1:30 PM-3:30 PM)

Title Nitric Oxide-Sensitive Guanylyl Cyclase α_1 - and β_1 -Subunits Are Differentially Regulated by Classical and Non-Classical Estrogen Pathways in Anterior Pituitary Gland

Author String JP Cabilla, SA Ronchetti, SI Nudler, FA Quinteros, BH Duvilanski
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Body 17 β -estradiol (E2) regulates hormonal release as well as pituitary cells' proliferation and death. The main nitric oxide receptor, soluble guanylyl cyclase (sGC), is a heterodimer composed by two subunits α and β and catalyzes cGMP formation. Previously we have shown that E2 exerts opposite effects on these sGC subunits, increasing both α_1 mRNA and protein levels and decreasing β_1 expression *in vitro* and *in vivo*. In the present work we investigate the mechanisms by which E2 through classical and/or non classical pathways regulates sGC subunits' expression on primary cultures of anterior pituitary cells. After 6 h, actinomycin D (2 [mu]M) partially abolished E2-elicited α_1 mRNA and protein augment and β_1 levels decrease (relative units (RU) as % of control (C): E2, α_1 : 148 \pm 11**, β_1 : 72 \pm 8*; Act D, α_1 : 91 \pm 7, β_1 : 94 \pm 6; E2+Act D, α_1 : 121 \pm 9#, β_1 : 86 \pm 8; *p<0.05, **p<0.01 vs C; #p<0.05 vs E2, n=3). Cycloheximide (10 [mu]g/mL) fully abolished E2 actions on α_1 and β_1 mRNA expression (RU as % of C; E2, α_1 : 162 \pm 13**, β_1 : 48 \pm 7**; CHX, α_1 : 98 \pm 5, β_1 : 102 \pm 4; CHX+E2, α_1 : 99 \pm 4###, β_1 : 100 \pm 5#; **p<0.01 vs C; #p<0.05, ###p<0.01 vs E2, n=3). E2 decreased HuR mRNA stabilization factor and increased AUF1p37 mRNA destabilization factor expression (% of respective C, HuR: 46 \pm 5**, AUFp37: 142 \pm 2**, **p<0.01, n=3). After 6 h of treatment, E2-BSA (1 nM) and E2-dendrimer conjugate (EDC, 1 nM) were unable to modify α_1 or β_1 mRNA levels, showing that intracellular receptor is involved in these E2 actions. However, at earlier times (3 h), 1 nM EDC decreased α_1 mRNA levels and slightly increased β_1 levels (RU as % of C; E2, α_1 : 115,6 \pm 10*, β_1 : 67 \pm 17*; empty dendrimer, α_1 : 95 \pm 24, β_1 : 114 \pm 5; EDC, α_1 : 59 \pm 10#, β_1 : 88 \pm 11; *p<0.05 vs C, #p<0.01 vs E2 n=4), opposite to E2 effects at longer times of exposure. Here we show for the first time that E2 activation of membrane or cytoplasmic pathways leads to different effects on sGC subunits expression. Upon membrane receptors activation, E2 causes a transiently decrease of α_1 subunit expression, contrary to that observed at longer times. At this time (6 h), α_1 levels are increased, E2 effects on sGC expression is partially dependent on *de novo* transcription and *de novo* translation is fully required. E2-elicited β_1 mRNA downregulation correlates with mRNA destabilization environment in anterior pituitary gland. Our results show that E2 has an opposite effect on sGC expression depending on the activation of classical or non-classical pathways.

Sources of Research Support: Universidad de Buenos Aires grant B052; CONICET grant PIP 112-200801-01977; Acknowledgments: John A Katzenellenbogen and Sung Hoon Kim, NIH grant R37 DK015556 for kindly providing EDC compound.

Nothing to Disclose: JPC, SAR, SIN, FAQ, BHD

Pub # P3-23

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Molecular & Physiological Actions of the Sex Steroid Receptors (1:30 PM-3:30 PM)

Title The Role of Tamoxifen-Induced Signaling in the Suppression of Malignant Mesothelioma Cell Growth

Author String CJ Jennings, T O'Grady, E Kay, BJ Harvey, W Thomas
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Body Malignant Mesothelioma (MM) is an aggressive tumor with extremely poor prognosis that most often arises from the pleural mesothelium of the lungs. Exposure to asbestos fibres is the greatest risk factor for MM development; even so the malignancy is usually diagnosed more than twenty years after initial exposure. The incidence of MM is reaching a peak in the majority of developed nations; however, the incidence in developing countries is predicted to rise for decades to come. Female gender is a positive prognostic indicator for MM progression and post diagnosis survival, which has been attributed to hormonal effects influenced by expression of estrogen receptor (ER) β isoform (1, 2). Tamoxifen has partial agonist/antagonist activity on ER β and was used in conjunction with cisplatin in a clinical study, where it was found to have beneficial results in a small subset of MM patients treated (3). In this present study the expression of ER α and ER β in MM tumor samples was investigated by immuno-histochemistry using ER isoform-specific antibodies and ER expression by six MM cell lines was determined by Western blotting. MTS cell viability assays were performed on MPP-89 (Epithelioid), REN (Epithelioid) and MSTO-211H (Biphasic) MM cell lines subjected to treatment with varying concentrations of either tamoxifen (0.1 to 10 μ M) or cisplatin (0.1 to 50 μ M) and also with tamoxifen (5 μ M) and cisplatin (0.1 to 50 μ M) in combination for up to 72h. Immuno-histochemistry and Western blotting showed that MM cell lines and tumor tissue expressed ER β and not ER α . Cell viability assays demonstrated a significant decrease in viability for all three MM cell lines at tamoxifen concentrations above 5 μ M after 72h. When cisplatin and tamoxifen were used in combination, there was no additive effect on reducing cell viability for the MPP-89 and REN cell lines; however, tamoxifen co-treatment (5 μ M) decreased the cisplatin-sensitivity of the MSTO-211H cell line. Modulation of ER-coupled signalling may provide novel approaches to therapeutic intervention for this terminal malignancy; however, tamoxifen is likely to have tumor promoting effects when used in combination with standard MM chemotherapeutic interventions in some patients.

- (1) Pinton G et al., Cancer Res 2009; 69:45982
- (2) Pinton G et al., PLoS One 2010; 5:e14110
- (3) Pass H W et al., Ann Surg Oncol 1995; 2:214

Sources of Research Support: Health Research Board of Ireland and by the Higher Education Authority of Ireland through the National Biophotonics and Imaging Platform.

Nothing to Disclose: CJJ, TO, EK, BJH, WT

Pub # P3-24

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Molecular & Physiological Actions of the Sex Steroid Receptors (1:30 PM-3:30 PM)

Title Ligand-Independent Transcriptional Actions of Human ER β Splice Variants on ERE, AP-1 and Human Arginine Vasopressin (hAVP) Promoters in Neuronal Cells

Author String NN Mott, TR Pak
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Body Estrogens are critical regulators of many CNS functions including homeostasis, reproduction, learning, and memory. The actions of both endogenous and synthetic estrogens are mediated primarily by two high-affinity estrogen receptors belonging to the family of steroid hormone receptors (SHRs), ER α and ER β . SHRs are comprised of five functional domains, an AF-1 containing region (Domain A/B), DNA binding domain (C), hinge region (D) and a ligand binding domain (E). ERs contain a unique sixth domain (F), the function of which remains unclear. Also, ER β has several splice variant isoforms present in the brain of both rat and human. The human isoforms identified to date contain variable length deletions and substitutions in exons 7 and/or 8 (e.g. ER β 1, ER β 2, ER β 4, and ER β 5), resulting in truncated receptor proteins which are missing the F domain and contain an altered AF-2 pocket at the C-terminus. By contrast, the rat isoforms contain deletions in exons 3 or 4 (e.g. ER β 1[Delta]3 and ER β 1[Delta]4), and also amino acid insertions in the ligand binding domain (e.g. ER β 2). Previously, we showed, in neuronal cells, that rat ER β 1 increased transcriptional activity mediated by an estrogen response element (ERE), and at a complex promoter for the neuropeptide arginine vasopressin (AVP). However, rat ER β 1 induced a constitutive decrease in transcriptional activity mediated at the activator protein-1 (AP-1) site. Rat and human ER β are highly homologous, therefore in this study we used the human splice variants of ER β in order to determine the relative importance of the E and F domains for conferring ligand-independent activity when mediated by an ERE or AP-1 site, and at the human AVP promoter. Our results reveal dose-dependent ligand-independent activation of all human splice variants at a tandem ERE site as well as dose-dependent ligand-independent repression of AP-1 mediated promoter activity. The human splice variants also exhibit ligand-independent repression of the human AVP promoter, which was mediated through AP-1 activity. These data provide further understanding about the precise functions of each ER β isoform in the brain, and highlight similarities and differences between the rat and human ER β isoforms. Moreover, these data provide insight into the relative contributions of the six functional domains of ER β towards mediating transcription at various cis-regulatory promoter regions.

Sources of Research Support: NIA RO1AG033605-01; NIH T32 AG031780 awarded to NNM.

Nothing to Disclose: NNM, TRP

Pub # P3-25

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Molecular & Physiological Actions of the Sex Steroid Receptors (1:30 PM-3:30 PM)

Title Identification of Gestationally Regulated Estrogen Receptor α (ESR1) Variants in the Pregnant Uterus

Author String RC Moore, C Kachulis, A Suresh, SN Caritis, JC Condon, P Jeyasuria
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Body In this study we have identified alternatively spliced ER α transcripts that give rise to ER α 51 and ER α 46 protein variants in the pregnant human and mouse uterus. Due to their abundance and gestationally regulated expression pattern we propose that the truncated variants may play a critical role in governing uterine ER α 66 action during pregnancy. Previous studies have identified both ER α 46 and ER α 51 as potential modifiers of both estrogen and ER α 66 action.

Utilizing human myometrial tissues isolated from term and pre-term non-laboring patients, a hTERT myometrial cell line and a gestational series of pregnant mouse uteri we identified by western blot analysis two alternate ER α isoforms, ER α 46 and ER α 51. To validate the presence of the ER α protein variants we undertook RNase protection assay (RPA) and RTPCR analysis on the same tissues. RTPCR was performed to determine the exon usage in the uterine cells by utilizing primers that span all the 8 exons. Sequencing of the PCR products revealed a common splice variant in the ER α mRNA in which exon 7 was absent giving rise to the truncated 51 kDa ER protein ([Delta]7 isoform). RTPCR analysis utilizing primers spanning the known 5' untranslated regions and exon 8 was performed and PCR products from this analysis were sequenced. Sequence data revealed both the full length ER mRNA and a splice variant where exon 1 is skipped and an alternate start site in exon 2 is utilized giving rise to the truncated 46 kDa ER protein ER α 46. RPA confirmed that the ER α 51 isoform was as abundant as the mRNA that gives rise to the full length 66 kDa ER protein, ER α 66.

In human myometrial tissues, ER α 46 is present in the nucleus whereas ER α 51 is found both in the nucleus and cytoplasm while the classical ER α 66 is restricted to the cytoplasmic fraction in pregnancy. ER α 51 protein expression is higher in human preterm pregnant myometrium when compared to term pregnant myometrium. In both human and mouse uterine tissues we observe elevated levels of the truncated ER isoforms at earlier gestational time points. These isoforms diminish as term approaches. We hypothesize that the truncated forms of ER may inhibit ER α 66 action earlier in gestation but as term approaches and their levels decline, this may allow increased uterine ER α 66 action and the onset of labor.

Sources of Research Support: HD047905.

Nothing to Disclose: RCM, CK, AS, SNC, JCC, PJ

Pub # P3-26

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Molecular & Physiological Actions of the Sex Steroid Receptors (1:30 PM-3:30 PM)

Title Development of an Animal Model of Estrogen-Dependent Ovarian Epithelial Cancer

Author String MJ Laws, A Kannan, S Pawar, MK Bagchi, IC Bagchi
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Body Ovarian epithelial cancer is the most lethal malignancy of the female reproductive system because the majority of the afflicted women are diagnosed at a late stage of the disease. The etiology of ovarian epithelial cancer is poorly understood, mainly due to the lack of an appropriate experimental model for studying the onset of this disease.

We have created a mouse model in which aberrant estrogen receptor alpha (ER α) signaling in the hypothalamo-pituitary-ovarian axis leads to ovarian tumorigenesis. In this model, termed ER α d/d, the ER α gene is conditionally deleted in the anterior pituitary, but remains intact in the hypothalamus and the ovary. The ER α d/d mice exhibited formation of palpable ovarian tumors, starting at 5 months of age. By 8 months, these tumors grew up to 11 mm in size and, by 12 months, most mice carrying these tumors died. The tumors are characterized by expression of cytokeratin 8 and 19 as well as nuclear staining of Wilms tumor 1 and Paired Homeobox 8, well-known markers of ovarian epithelial tumors. Besides proliferating epithelial cells, these tumors also contained an expanded population of stromal cells.

The selective ablation of ER α in the anterior pituitary of ER α d/d mice resulted in a systemic hormonal imbalance. The loss of negative-feedback regulation by estrogen (E) at the level of the pituitary led to elevated production of luteinizing hormone (LH) by this tissue. Hyperstimulation of ovarian cells by LH resulted in increased steroidogenesis, leading to high circulating levels of progesterone, testosterone and E. Analyses of ovarian tumor cells isolated from ER α d/d mice revealed a high level of P450 aromatase expression in the stromal cells of the tumor, suggesting that these cells acquired the ability to synthesize E. In ER α d/d mice, in response to the E produced by the stromal cells, the ER α signaling is accentuated in the ovarian epithelial cells, triggering increased ER α -dependent gene expression, abnormal cell proliferation, and tumorigenesis. To confirm this hypothesis, we placed an implant of letrozole, a specific inhibitor of P450 aromatase, in ER α d/d mice. Remarkably, ovarian tumors of ER α d/d mice treated with letrozole displayed about 60% reduction in tumor volume when compared to untreated controls. We have, therefore, developed an animal model of ovarian epithelial tumorigenesis, which will serve as a powerful tool for exploring the involvement of E-dependent signaling pathways in the etiology of this cancer.

Nothing to Disclose: MJL, AK, SP, MKB, ICB

Pub # P3-27

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Molecular & Physiological Actions of the Sex Steroid Receptors (1:30 PM-3:30 PM)

Title Control of Progesterone Receptor (PR) Transcriptional Activity by SUMOylation and De-SUMOylation

Author String HA Abdel-Hafiz, ML Dudevair, KB Horwitz
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Body Covalent modification of nuclear receptors and co-factors by the Small Ubiquitin-like Modifier (SUMO) has an important role in modulating their overall transcription activity. SUMOylation is a highly dynamic process controlled by competing conjugating and deconjugating steps. We have shown that SUMO conjugation of progesterone receptors (PR) at K388 is hormone-dependent, and suppresses PR-dependent transcription of promoters containing multiple progesterone response elements (PREs), but not single PRE. The ligand binding domain of PR is required for SUMOylation at K388, but a functional DNA binding domain is not. The mouse mammary tumor virus (MMTV) promoter, commonly used to study PR-dependent transcription, contains 1 PRE and 3 half-sites. Of the two PR isoforms, PR-A has only modest effects on MMTV, whereas PR-B is a potent activator. However, PR SUMOylation does not control transcription of MMTV, suggesting instead a role for the AF3 in the far N-terminus of PR-B to positively control receptor-loading synergy on both compound promoters and MMTV. Interestingly, activation of MAPK signaling with a constitutively active MEKK blunts SUMOylation of both wild-type PR and MAPK phosphorylation PR mutants, suggesting that the attenuating effects of MAPK signaling on PR SUMOylation are indirect, *via* changes in the activity of the general SUMOylation machinery. SUMO is deconjugated from the receptors by SUMO-specific proteases (SENPs). PR transcriptional activity is markedly coactivated by SENP1 and SENP2-dependent de-SUMOylation, in a promoter-specific manner with SENP1-stimulating PR activity on compound PRE-containing promoters but not on MMTV. The effect of SENP requires that its catalytic activity be intact, and that the K388 SUMO conjugation site in PR be intact. This indicates that the coactivating effects of SENP are mainly due to their isopeptidase properties. Our results indicate that SENP1, but not a constitutively active MEKK, enhances PR transcriptional activity by reversing ligand-induced SUMOylation of PR.

Nothing to Disclose: HAA-H, MLD, KBH

Pub # P3-28

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Molecular & Physiological Actions of the Sex Steroid Receptors (1:30 PM-3:30 PM)

Title C/EBP β Regulation of Progesterone Receptor Expression

Author String W Wang, MD Aupperlee, S Durairaj, E Flynn, A Drolet, RJ Miksicek, SZ Haslam, RC Schwartz Michigan State University, East Lansing, MI; Michigan State University, East Lansing, MI

Body C/EBP β is a critical transcription factor in the regulation of mammary gland proliferation and development. Experiments with C/EBP β knockout mice demonstrate a requirement of C/EBP β for ductal morphogenesis and alveologenesis. The overall decrease in progesterone receptor (PR) observed in sexually mature wild type mice also fails to occur in C/EBP β knockout mice. Progesterone is a critical factor for proliferation and alveologenesis in the adult mammary gland, and experiments with PRB knockout mice show a requirement of PRB for alveologenesis. Not only do C/EBP β and PRB knockouts show similarly defective alveologenesis, but the PR promoter region has multiple C/EBP consensus binding sites. We therefore hypothesized that C/EBP β can regulate PRB expression. C/EBP β occurs in three isoforms in mammary and other tissues: C/EBP β p39 (LAP-1) and C/EBP β p36 (LAP-2), both potent transcriptional activators, and C/EBP β p20 (LIP), a truncated form generally reported to inhibit C/EBP-dependent transcription. Transient cotransfection of PR promoter-reporter constructs with expression vectors that individually express C/EBP β isoforms into a mammary tumor cell line revealed that all C/EBP β isoforms, surprisingly including LIP, could transactivate the PR promoter. Analysis of C/EBP β dimerization suggested that LAP-1/LIP heterodimers are the most potent form of C/EBP β in PR transcription. Furthermore, all three C/EBP β isoforms favored the transcription of PRB over PRA. We also found that LIP synergized with c-Jun to drive PR transcription, perhaps explaining the potency of LAP-1/LIP heterodimers. In the course of pregnancy, both PRB and the relative abundance of LIP among C/EBP β isoforms increase. Consistent with a role in the expression of PRB, in vivo immunofluorescence studies showed that C/EBP β and PRA expression are mutually exclusive in mammary epithelium, while PRB is only expressed in cells that express C/EBP β . Collectively, our data suggest a critical role for C/EBP β , particularly LIP, in PRB expression.

Sources of Research Support: Breast Cancer and the Environment Research Centers Grant U01 ES/CA 012800 from the National Institute of Environment Health Science (NIEHS) and the National Cancer Institute (NCI), National Institutes of Health (NIH), Department of Health and Human Services.

Nothing to Disclose: WW, MDA, SD, EF, AD, RJM, SZH, RCS

Pub # P3-29

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Molecular & Physiological Actions of the Sex Steroid Receptors (1:30 PM-3:30 PM)

Title Overactive AKT and MAPK Pathways Promote Progesterone Receptor Degradation in Endometriosis

Author String JL Eaton, JJ Kim
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Body Endometriosis is considered to be a progesterone resistant disease. We and others have observed hyperactivity of the AKT and mitogen-activated protein kinase (MAPK) pathways in endometriotic stromal cells. Given that progesterone receptor (PR) is heavily regulated at the post translational level by signaling pathways and that PR levels are low in endometriosis, we hypothesized that the overactive AKT and MAPK pathways contribute to PR degradation. Stromal cells were isolated from human ovarian endometriomas or normal endometrial tissue and cultured. The cells were pretreated with either the AKT inhibitor MK-2206 (100 nM), the MEK1[frasl]2 inhibitor U0126 (10 [mu]M), or vehicle for 2 hours. They were then incubated for 24 hours with or without the progestin R5020 (100 nM). Whole cell lysates or nuclear and cytoplasmic lysates were obtained, and PR levels were determined by Western blot. Levels of phospho-AKT or phospho-ERK decreased following treatment with MK-2206 or U0126, respectively, while total AKT and ERK levels were unchanged. In the absence of R5020, treatment with MK-2206 or U0126 increased total and nuclear PRA and PRB protein. These effects were not seen in normal endometrial stromal cells. Treatment with R5020 decreased whole cell PRA and PRB protein levels in both endometriotic and normal endometrial stromal cells these levels were not rescued by treatment with MK-2206 or U0126. To determine whether MK-2206 or U0126 treatment influenced PR expression at the transcriptional level, mRNA was obtained from endometriotic stromal cells and quantitative RT-PCR performed for total PR (PRA and PRB) or PRB. Total PR and PRB mRNA levels did not significantly change among the treatment groups, with the exception of a decrease in PRB mRNA with R5020 and U0126. Cells were then treated with the proteasome inhibitor MG132 (20 [mu]M) or vehicle for 30 minutes, followed by 24 hour incubation in the presence or absence of R5020 (100 nM). Whole cell lysates were obtained and PR protein levels determined by Western blot. In the absence of ligand, MG132 increased PRB. Treatment with MG132 in the presence of R5020 rescued PRA and PRB. These data suggest that proteolysis contributes to the decreased PR levels in endometriotic stromal cells and that inhibition of AKT or MEK1[frasl]2 increased PR protein stability in the absence of ligand. Additional research is needed to determine whether the increased PR levels are transcriptionally functional.

Nothing to Disclose: JLE, JJK

Pub # P3-30

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Molecular & Physiological Actions of the Sex Steroid Receptors (1:30 PM-3:30 PM)

Title Progesterone Receptor Membrane Component 1 and 2 in Normal and Malignant Endometrial Cells

Author String L Kavandi, VS Ivanova, H Nguyen, V Syed
Uniformed Services University of the Health Sciences, Bethesda, MD

Body Recently, two progesterone receptor membrane components (PGRMC) have been identified. PGRMC-1 protein has been shown to be overexpressed in breast, colon, and thyroid cancers, whereas far less is known about PGRMC2. The purpose of this study was to assess the expression, localization and hormonal regulation of PGRMC-1 and PGRMC-2 in normal and malignant endometrial tissues and cell lines. Immunohistochemical analysis showed a high expression of PGRMC-1 in normal tissues and a stage dependent decrease was observed in endometrial cancer tissues. A progressive decrease of PGRMC-2 expression was evident in endometrial cancer tissues but was not as marked as in PGRMC-1. Dual immunofluorescent confocal microscopy demonstrated colocalization of PGRMC-1 and PGRMC-2 in the cytoplasm and nucleus of endometrial cancer (Ishikawa and ACI-126) cells. Of significance, in normal epithelial endometrial (NEE) cells PGRMC-2 was predominantly localized in the membrane, whereas PGRMC-1 showed cytoplasmic and nuclear expression. Treatment of cancer cells with progesterone (P4) translocated both PGRMCs to the nucleus, while in normal cells PGRMC-1, but not PGRMC2 was translocated to the nucleus of cells. The results suggest that expression of PGRMCs is regulated by P4 and the dynamic pattern of PGRMC1 and PGRMC2 expression in normal and malignant cells implicate their role in tumorigenesis.

Nothing to Disclose: LK, VSI, HN, VS

Pub # P3-31

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Molecular & Physiological Actions of the Sex Steroid Receptors (1:30 PM-3:30 PM)

Title Decreased Tumorigenesis, Growth, and Mortality of Chemical Carcinogen-Induced Bladder Cancer in Mice Lacking Urothelial Androgen Receptor

Author String J-W Hsu, H Miyamoto, C Chang
University of Rochester, Rochester, NY

Body Males have a higher incidence of bladder cancer (BCa) than females. Our previous study using general AR knockout (ARKO) mice suggested the involvement of both androgens and androgen receptor (AR) in BCa development. However, the role of urothelial AR in BCa development remains unclear. To address this question, we generated urothelial ARKO (U-ARKO) mice and induced BCa using the chemical, N-butyl-N-(4-hydroxybutyl)-nitrosamine (BBN). In this BBN-induced BCa model, U-ARKO mice showed a lower tumor incidence than their wild-type littermates ($p < 0.05$). Immunohistochemistry data revealed that U-ARKO urothelium expressed less DNA damage than wild-type urothelium, implying that urothelial AR may regulate bladder tumorigenesis through DNA damage repair mechanisms. Indeed, U-ARKO urothelium had higher levels of p53, the protein which regulates DNA damage repair and bladder tumorigenesis. Moreover, U-ARKO urothelium expressed more PCNA, which also plays a role in DNA damage repair and is transactivated by p53. To determine if AR regulates bladder tumorigenesis through p53, we generated U-ARKO mice with transgenic UPII-SV40T, in which SV40T antigen is only expressed in urothelium. In this model, BCa spontaneously develops due to the blocking of p53 by the SV40T antigen. As we expected, no difference in BCa incidence was noted between wild-type and U-ARKO mice with UPII-SV40T, suggesting that urothelial AR regulates bladder tumorigenesis through p53. The modulation of p53/PCNA signaling by AR was also confirmed by *in vitro* data. To study the role of AR in BCa growth, we examined the growth of the BBN-induced bladder tumor tissues from 30-week-old wild-type and U-ARKO mice. U-ARKO tumor tissues expressed a lower proliferation index and a higher apoptosis index than wild-type tissues. Reversely, the UPII-SV40T mice model showed no difference in tumor growth between wild-type and U-ARKO, which suggests that urothelial AR may contribute to BCa growth through p53 as well. Lastly, we assessed the survival rates of BBN-treated wild-type vs. U-ARKO mice. The U-ARKO mice showed a higher survival rate than wild-type mice ($p < 0.05$). Together, we provide the novel finding that urothelial AR modulates bladder tumorigenesis, BCa growth, and survival. We further dissect the detailed mechanisms by which urothelial AR promotes BCa development through p53. Our results may provide a solid rationale for potential therapeutic strategies of BCa by targeting urothelial AR.

Nothing to Disclose: J-WH, HM, CC

Pub # P3-32

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Molecular & Physiological Actions of the Sex Steroid Receptors (1:30 PM-3:30 PM)

Title Differential Regulation of Androgen Receptor Biology by the Homeobox Protein, HOXB13

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Body Androgen Receptor (AR) is a ligand-activated transcription factor that responds to androgens by systematic activation and repression of subsets of genes, which is tightly regulated and fine-tuned by a large class of proteins called coregulators. Recently, our lab characterized a prostate-specific transcription factor, HOXB13, as an important coregulator of AR in prostate cancer cells. HOXB13 is a key regulator that maintains a balance between cell growth/proliferation and more differentiated functions like lipogenesis (1). In this study, we hypothesized that HOXB13 regulates AR signaling by distinct and separable mechanisms that drive androgen-responsiveness of prostate cancer cells. Initially, we investigated the interaction between AR and HOXB13, by creating various deletion mutants of HOXB13 to map the AR interaction region. We identified a 42 amino acid (aa) region (174-215 aa) within HOXB13 that constitutes the AR-interaction region, which is conserved among the group-13 homologues of HOX proteins. To further resolve the minimal AR-interaction region of HOXB13 we substituted every 6 residues of this 42 aa region with a NAAIRS (single letter amino acid code) sequence in the full length HOXB13 protein. Surprisingly, these individual NAAIRS substitutions had no significant impact on HOXB13:AR interaction, indicating that the 42 aa stretch may encompass multiple contact region between AR and HOXB13. Next, we explored how this interaction region impacts the overall functions of HOXB13. Our data indicates that these mutants had significantly different functional profile; mutations spanning residues 186-191 or 204-209 of the HOXB13 protein rendered it functionally dead, whereas mutation of 174-179, 180-185, 198-203 and 209-215 had no effect on HOXB13-mediated activation or repression. Interestingly, the HOXB13 mutant carrying NAAIRS substitution of residues 192-197 retained its repressive effect on AR but lost its ability to function as an AR coactivator (on reporter gene assays). Our data also suggests that this region may facilitate interaction with other yet-to-be identified protein(s) that may be functionally relevant for HOXB13-mediated co-activation of AR. Taken together these observations are consistent with our overall hypothesis but these HOXB13 mutants warrants further characterization to test whether they can drive selective AR-responsive processes like proliferation versus migration, invasion, lipogenesis, etc, prostate cell models.

1. Norris, J.D., et al., The homeodomain protein HOXB13 regulates the cellular response to androgens. *Mol Cell*, 2009. 36(3): p. 405-16.

Nothing to Disclose: SCD, DPM

Pub # P3-33

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Molecular & Physiological Actions of the Sex Steroid Receptors (1:30 PM-3:30 PM)

Title High Content Analysis of an Androgen Receptor Splice Variant Found in Late-Stage Prostate Cancer

Author String AT Szafran, S Mediwala, M Marcelli, M Mancini
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Body AR-V7 is an androgen receptor (AR) splice variant isolated from prostate cancer cell lines. AR-V7 is expressed at low levels in normal/benign prostate tissue and at high levels in advanced, late stage castration resistant prostate cancer (CRPC). AR-V7 lacks the C-terminal ligand binding domain (LBD) and has been shown to be constitutively nuclear and transcriptionally active in the absence of hormone, suggesting a potential role in CRPC. Since all AR antagonists commonly used target the LBD, they are ineffective at inhibiting AR-V7 activity. To begin to understand signaling pathways that may be able to regulate the outlaw activity of AR-V7 at a systems biology level, we have used AR high content analysis (AR-HCA) (Szafran et al, 2008, 2009) to characterize the AR-V7 response to a small panel of kinase inhibitors and AR ligands, including MDV3100. AR-HCA was done with a PC-3 prostate cancer cell line stably expressing GFP-AR-V7 and transiently-transfected with either an AR responsive probasin reporter construct driving expression of a nuclear mCherry fluorescent protein (ARR2PB-mCherry-NLS) or a FLAG tagged wild type AR (wtAR) expression vector. Four color images were analyzed using in-house developed Pipeline Pilot (Accelrys) algorithms that simultaneously determine AR-V7 transcriptional activity, expression level, and subcellular and subnuclear distribution on a cell-by-cell basis. As expected, the LBD targeting compounds dihydrotestosterone (DHT), R1881, o-hydroxyflutamide, and bicalutamide did not have a significant effect at 48 hrs. Interestingly, the novel AR inhibitor MDV3100 was able to induce a minor cytoplasmic shift (9 % shift, $p = 0.03$) in AR-V7, however, only in the presence of wtAR. In contrast, the MAPK inhibitor UO126 resulted in a significant decrease in AR-V7 transcriptional activity (46%, $p < 0.01$), nuclear hyperspeckling (43%, $p < 0.01$), and nuclear accumulation of AR-V7 (20% shift, $p < 0.01$). These effects were not dependent on wtAR expression. Further IP-mass spectrometry analysis of AR-V7 following UO126 treatment demonstrated an altered protein interaction profile and suggest a potential role for N-terminal methylation sites. AR HCA was also used to profile a focused library of clinical/preclinical small molecule inhibitors and identified an additional ~20 compounds with significant affects on AR-V7. These studies suggest that AR-V7 constitutive activity can be altered through manipulation of its residual NTD.

Nothing to Disclose: ATS, SM, MM, MM

Pub # P3-34

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Molecular & Physiological Actions of the Sex Steroid Receptors (1:30 PM-3:30 PM)

Title Genome-Wide Analysis of Androgen Receptor Binding Sites in Mouse Prostate

Author String P Pihlajamaa, B Sahu, V Aittomaki, S Hautaniemi, OA Janne
University of Helsinki, Helsinki, Finland; University of Helsinki, Helsinki, Finland

Body Androgen receptor (AR) is a ligand-inducible transcription factor that mediates the actions of male sex hormones. To study genome-wide AR binding to chromatin in vivo, we performed ChIP-sequencing using polyclonal anti-AR antibody from prostates of castrated mice exposed to testosterone (1 mg, sc) for 2 h. Two testosterone-treated samples were sequenced, and only the peaks present in both biological replicates were considered significant for downstream analyses. DNA immunoprecipitated with anti-AR antibody from castrated mouse prostate was used as a negative control for peak finding. By using a stringent false discovery rate of 0.02, we found 6,618 AR binding sites (ARBs) in mouse prostate. The distribution of these sites was in agreement with those in cell lines, in that most of the sites (57%) are located in distal enhancer regions, and only a minority of them (2.5%) is enriched to the proximal promoter region. About one third (35%) of the binding sites are in intronic regions. De novo motif search revealed that a canonical androgen response element (ARE) sequence was enriched among the ChIP-seq peaks. To identify androgen-regulated transcripts in mouse prostate, gene expression profiling was performed from prostates of intact and castrated males. With a fold-change >1.5 cut-off, expression of 743 transcripts was decreased and 653 transcripts increased in response to androgen withdrawal, and these transcripts were considered androgen up-regulated and androgen down-regulated, respectively. Correlation of expression profiling results to ChIP-seq data revealed that 71% of the androgen up-regulated and 53% of androgen down-regulated genes have at least one ARB within ± 100 kb of the transcription start site. Androgen up-regulated genes with an ARB belong to Gene Ontology (GO) categories related to cellular metabolism, including carbohydrate metabolism and amino acid biosynthesis. GO categories such as immune system process, leukocyte activation and developmental process were enriched among the androgen down-regulated genes. Motif enrichment analysis was performed separately for ARBs associated with androgen up-regulated and down-regulated genes, and a canonical ARE was present in 40% and 20% of them, respectively. Interestingly, motifs for forkhead family transcription factors were significantly over-represented in close proximity of the ARBs of androgen up-regulated, but not of androgen down-regulated genes.

Sources of Research Support: CRESCENDO; Sigrid Juselius Foundation; Academy of Finland.

Nothing to Disclose: PP, BS, VA, SH, OAJ

Pub # P3-35

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Molecular & Physiological Actions of the Sex Steroid Receptors (1:30 PM-3:30 PM)

Title Investigating Non-DNA Binding-Dependent Signaling Pathways of the Androgen Receptor

Author String TPS Pang, RA Davey, MV Clarke, JPJ Skinner, K Rana, NKL Lee, HE MacLean
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Body Ligand-bound androgen receptor (AR) binds DNA and directly regulates gene expression (genomic action), but may also have non-DNA binding-dependent actions, including indirect gene repression occurring in the nucleus [1] and activation of ERK/CREB signaling in cytoplasm [2]. The aim of this study is to investigate the non-DNA binding-dependent actions using our DBD-ARKO mouse model, which has an in-frame deletion of the 2nd zinc finger of the DNA-binding domain [3]. We have previously shown this model has deletion only of the DNA binding-dependent genomic AR actions and the mutant AR binds ligand normally [4]. Immunocytochemistry and Western analyses showed that the mutant AR has reduced nuclear translocation following ligand-binding in cultured genital skin fibroblasts (GSFs), suggesting the nuclear non-DNA binding-dependent actions may be attenuated in DBD-ARKOs. Quantitative real-time PCR (Q-PCR) confirmed this by showing non-DNA binding-dependent AR target genes *Ngfr* [1] and *Mmp13* [5], which are repressed by the AR in the absence of direct DNA binding, are repressed following 10 nM dihydrotestosterone (DHT) treatment in wildtype (WT) but not DBD-ARKO GSFs. Both WT and DBD-ARKO GSFs have increased ERK phosphorylation following 1 min 100 nM DHT treatment ($p < 0.05$) and this is abolished by the AR antagonist bicalutamide. This indicates that the DHT-induced ERK phosphorylation is not dependent on DNA binding and is activated normally in DBD-ARKOs. *In vivo*, we have examined effect of 10 weeks orchidectomy \pm DHT replacement in WT and DBD-ARKO male mice. As expected, the genomic AR target gene, *Odc1*, is 5-fold up-regulated by DHT in WT but not DBD-ARKO kidney. Consistent with the *in vitro* data, *Mmp13* is repressed 1.7-fold by DHT in WT ($p < 0.05$) but not DBD-ARKO bone. Both CREB phosphorylation and expression are repressed by DHT in WT but not DBD-ARKO fat. DHT treatment increases the mass of heart, kidney and muscle and decreases spleen mass of spleen in WT only, demonstrating that these actions require binding of AR to DNA. No effect of DHT treatment on organ mass has been observed in DBD-ARKOs. We are currently determining the effects of DHT treatment on bone histomorphometric parameters and phosphorylation of other 2nd messengers in target tissues of DBD-ARKO males to determine if physiological actions of non-DNA binding-dependent AR pathways occur.

- (1) Kallio PJ et al., J Mol Endo 1995; 9:1017-1028
- (2) Unni E et al., Cancer Research 2004; 64: 7156-7168
- (3) Notini AJ et al., J. Mol Endo 2005; 35:547-555
- (4) Pang TPS et al., The US Endocrine Society 92nd Annual Scientific meeting 2010; p.12, P1-32
- (5) Schneikert J et al., J Biol Chem 1996; 271:23907-23913

Nothing to Disclose: TPSP, RAD, MVC, JPJS, KR, NKLL, HEM

Pub # P3-36

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Endocrine Neoplasia (1:30 PM-3:30 PM)

Title Clonality Analysis and Expression of ACTH and Androgen Receptors in Giant Myelolipomas

Author String LC Kaupert, L Brito, AM Lerario, BP Marinho, M Ribeiro, JV Lima Junior, O Monte, BB Mendoca, TASS Bachega
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Body **Introduction:** Adrenal myelolipoma is a rare nonfunctional benign tumor, usually unilateral and with unclear pathogenesis. Prolonged ACTH or androgen stimulation is suggested to take part in the etiology as they are also identified in Cushing[acute]s disease or CAH. Only one study evaluated ACTH (*MC2R*) and androgen (*AR*) receptor expression levels in giant myelolipoma of a CAH patient, not disclosing a role of stimulatory effects. **Objectives:** to evaluate *MC2R* and *AR* gene expression levels and clonality in giant bilateral adrenal myelolipomas from 2 females with simple virilizing CAH (P1/P2) and from 2 females with sporadic myelolipomas (P3/P4). **Patients and Methods:** abdominal masses of P1, 2, 3 and 4 were studied. P1 stopped treatment for 15 yrs and P2 was never treated. ACTH levels were available in P1 (1172 pg/mL) and testosterone levels >700 ng/dL in P1/P2. Giant bilateral adrenal myelolipomas were identified by CT in P1/P2 (> 20 cm) and unilateral in P3/P4 (8-10 cm). Histopatological studies confirmed myelolipoma diagnosis in all cases. Myelolipoma clonality was evaluated by X-inactivation pattern by *AR* CAG tract analysis. Tumor *MC2R*, *AR*, *Leptin*, *SF1*, β -actina and GAPDH mRNA levels were determined by Taqman real-time PCR using the $2^{-[\Delta\Delta]CT}$ method, with adipose tissue pool as calibrator. Adrenal tissue pool was studied to compare tumor adrenal contents. **Results:** All tumors were informative, CAG repeats varied from 20 to 30, except in P1 who carried a short allele (16 CAG). X-inactivation pattern analysis disclosed polyclonal origin in all tumors. While in samples from adrenal pool, P1 and P3, *AR* and *Leptin* mRNA levels were low, the opposite was found in P4. *MC2R* and *SF1* mRNA levels were high in samples from adrenal pool, P3 and P4, whereas normal expression levels were found in P1. *MC2R* and *AR* mRNA were expressed in P2, but mRNA quality was impaired. **Discussion:** *SF1* and *Leptin* mRNA levels observed in myelolipomas were variable, reflecting different adipose/adrenal tissue proportions. We identified high *MC2R* expression in sporadic myelolipomas. Despite P1 having normal *MC2R* expression, increased ACTH levels could have an additive effect. *AR* expression was low in P1 tumor, but an androgenic stimulatory effect cannot be excluded, since P1 had increased androgen levels and carried one short *AR* allele. **Conclusion:** we identified a polyclonal origin of myelolipomas and suggested a growth-stimulatory effect of ACTH and/or androgen pathways in patients with/without CAH.

Sources of Research Support: FAPESP # 08/57616-5, FAPESP for LCK # 08/55546-0, CNPq for TASSB # 305117/2009-2.

Nothing to Disclose: LCK, LB, AML, BPM, MR, JVLJ, OM, BBM, TASSB

Pub # P3-37

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Endocrine Neoplasia (1:30 PM-3:30 PM)

Title Utility of Basal DHEAS Measurement in the Detection of Subclinical Autonomous Glucocorticoid Hypersecretion in Adrenal Incidentaloma

Author String AK Annamalai, N Kandasamy, N Freeman, K Venugopal, J Chia, DJ Halsall, HL Simpson, AS Shaw, M Gurnell
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Body
Background: Adrenal incidentalomas (AI) are identified in 4-7% of patients >40y undergoing abdominal CT/MRI. Evidence of subclinical autonomous glucocorticoid hypersecretion (SAGH) is found in 5-10% of cases depending on the diagnostic criteria/thresholds adopted.
Aim: To examine the utility of basal dehydroepiandrosterone sulphate (DHEAS) measurement in the detection of SAGH in a cohort of patients with AI.
Methods: Ninety six consecutive subjects (49 female, 47 male: mean age 62y, range 29-84y) were referred to our clinic over a 3 year period. All patients were assessed by two clinicians (AA & MG), and underwent repeat dedicated adrenal imaging and biochemical profiling, including overnight dexamethasone suppression testing (normal cortisol suppression: <50nmol/L). Cases in whom there was no clinical or biochemical evidence of endocrine hyperfunction, and with appropriate radiological features, were categorised as non-functioning adrenal adenomas (NFAA). SAGH was confirmed by the finding of low ACTH levels, abnormal circadian rhythm, and failure of cortisol to suppress during a 48h low dose dexamethasone suppression test (normal: <50 nmol/L). DHEAS levels were classified as undetectable, suppressed (but detectable) and normal
Results: Amongst NFAA (n=41), DHEAS levels were normal in 38, suppressed in one, and undetectable in two subjects. Amongst SAGH (n=18) subjects, nine had undetectable DHEAS, three had suppressed, and six normal levels. Receiver Operating Characteristic analysis using DHEAS concentration to diagnose SAGH returned an Area Under the Curve of 0.85 (0.75-0.96). At the optimum cut-off of 0.9 nmol/L, sensitivity and specificity were 78% (52 to 94%) and 74% (58 to 87%) respectively. At a cut-point of 0.5nmol/L the test is specific (95%) with a sensitivity for the detection of SAGH of 50%.
Conclusion: The finding of a subnormal DHEAS level in a patient with an AI is an important pointer to the potential presence of SAGH and merits further investigation.

Nothing to Disclose: AKA, NK, NF, KV, JC, DJH, HLS, ASS, MG

Pub # P3-38

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Endocrine Neoplasia (1:30 PM-3:30 PM)

Title Comparable Inhibition of COLO205 Xenograft Tumor Growth by Nanoparticulate Tetraiodothyroacetic Acid (Nanotetrac) and Cetuximab

Author String M Yalcin, A Elsaid, E Dier, D Bharali, SA Mousa, FB Davis, H-Y Lin, PJ Davis
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Body Cetuximab (Erbix^{reg}) exhibits significant anti-tumor activity in a subset of patients with advanced colorectal cancer, reflecting inhibitory action of the monoclonal antibody on activity of the epidermal growth factor receptor (EGFR). Agonist thyroid hormone analogues induce proliferation of numerous human cancer cell lines via the cell surface receptor for the hormone on integrin $\alpha v \beta 3$. A deaminated analogue of L-thyroxine, tetraiodothyroacetic acid (tetrac), binds to the $\alpha v \beta 3$ integrin thyroid hormone receptor, thereby inhibiting the proliferative effects of the hormone. In certain tumor cells, tetrac may also suppress *EGFR* gene expression and modify EGFR activity. We determined the effectiveness of tetrac formulations and cetuximab, alone or in combination, against xenografts of human colon carcinoma (Colo205) cells. Effects of unmodified tetrac and nanoparticulate (poly-[lactate-co-glycolic acid])-tetrac (tetrac-NP) were studied for 8 d. Tetrac-NP acts exclusively at the hormone receptor on the integrin, whereas unmodified tetrac acts both at the cell surface and within the cell, where it is a low-grade thyromimetic. Duration of the study was determined by growth of the tumors in control animals to a size (2.0 gm) requiring animal sacrifice. Tetrac or tetrac-NP (0.1 mg tetrac/kg, ip, daily) were given, with or without cetuximab (0.1 or 1.0 mg/kg ip, qod). Tumor size and progression was measured daily with manual calipers. In preliminary studies, control tumors increased in volume by 64% (730 to 1200 mm³) in 2 d, whereas there was no increase in tumor volume in animals receiving 0.1 and 1.0 mg/kg of cetuximab or tetrac formulations. In the 8 d study, control tumors increased in volume by 2.7-fold from a baseline of 700 mm³. Administered individually, cetuximab and tetrac formulations, each at 0.1 mg/kg, permitted increases in tumor size of 0.4-fold to 0.7-fold, a mean reduction vs control of 80%. At the doses tested, co-administration of tetrac preparations with cetuximab did not have an additive or synergistic anti-tumor effect, whereas *in vitro* we have shown the agents to have an additive action (C Landersdorfer et al., PLoS Computational Biology, 2011, in press). Cetuximab and tetrac formulations, individually, are equally effective suppressors of human Colo205 xenografts. Absence of an additive effect in Colo205 cells is consistent with maximum action by each agent, in the concentrations used, primarily at the EGFR.

Nothing to Disclose: MY, AE, ED, DB, SAM, FBD, H-YL, PJD

Pub # P3-39

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Endocrine Neoplasia (1:30 PM-3:30 PM)

Title Anti-Metastasis Effect of Novel ALK5 Inhibitor in Both Breast Cancer Cells and 4T1-Luc Xenograft Mice

Author String JY Son, EJ Yoon, D-K Kim, YY Sheen
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Body We have synthesized novel ALK5 inhibitors and investigated their effects of on breast cancer cells as well as 4T1-Luc orthopathologically xenograft balbC mice. The treatment of ALK5 inhibitors to orthotopically injected mice inhibited the numbers of nodules in the lung compared to that of untreated animal. The level of β -casein as well as luciferase activity in the lung of 4T1-Luc orthotopically xenograft mice were decreased by ALK5 inhibitor treatment. Zymograph showed that the levels of MMP2 and MMP9 were decreased in the lung and breast tumor of ALK5 inhibitors treated breast tumor bearing MMTV/cNeu transgenic mice and 4T1 orthotopically xenograft mice. The action mechanisms of new ALK5 inhibitors were investigated in both breast cancer cells and breast cancer mice model. TGF β signaling, phosphorylated smad2/3 based from the western blot analysis in vitro and in vivo as well as confocal microscopic analysis in vitro was decreased by ALK5 inhibitors. And also, new ALK5 inhibitors inhibited the TGF β action on wound healing and cell invasion based on wound healing assay and invasion assay. This data strongly argue that ALK5 inhibitors have anti-metastasis effects via inhibiting ALK5 activity in vivo and in vitro. In order to gain further insight into the mechanism of ALK5 inhibitor, we have carried out experiment to examine the effects of ALK5 inhibitor in human breast cancer MDA-MB-231 cell. TGF β stimulated mesenchymal markers of epithelial mesenchymal transition (EMT), such as Snail, TWIST, N-cadherin and these were inhibited by ALK5 inhibitor concomitant treatment. Also, TGF β increased tumor invasion markers such as MMP2, and MMP9 which were inhibited by ALK5 inhibitor concomitant treatment. In summary, ALK5 inhibitors have anti-metastatic effect on human breast cancer cells as well as tumor bearing MMTV/cNeu transgenic mice.

Nothing to Disclose: JYS, EJY, D-KK, YYS

Pub # P3-40

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Endocrine Neoplasia (1:30 PM-3:30 PM)

Title Progesterone and Estrogen Receptor Expression and Biologic Activity in Human Non-Small Cell Lung Cancer

Author String DC Marquez-Garban, EL Maresh, M Alavi, H-W Chen, V Mah, L Bagryanova, HJ Garban, S Horvath, D Chia, EB Garon, L Goodglick, RJ Pietras
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Body Lung cancer is the most common cause of cancer mortality in male and female patients in the US. Although it is clear that tobacco smoking is a major cause of lung cancer, about half of all women with lung cancer worldwide are never-smokers. Despite a declining smoking population, the incidence of non-small cell lung cancer (NSCLC), the predominant form of lung cancer, has reached epidemic proportions particularly in women. Emerging data suggest that factors other than tobacco, namely endogenous and exogenous female sex hormones, have a role in stimulating NSCLC progression (1)(2). The results of recent prospective, randomized clinical trials also show that estrogen and progestins but not estrogens alone (3) enhance the mortality (4) and incidence (5) of lung cancer in postmenopausal women. Aromatase, a key enzyme for estrogen biosynthesis, is expressed in NSCLC. Clinical data show that women with high levels of tumor aromatase (and high intratumoral estrogen) have worse survival than those with low tumor aromatase (6). The present and previous studies also reveal significant expression and activity of estrogen receptors (ER α and ER β) in both extranuclear and nuclear sites in most NSCLC specimens from the clinic. We now report further on the expression of progesterone receptor (PR) transcripts and protein in NSCLC. PR transcripts were significantly lower in cancerous as compared to non-malignant tissue (P<0.01). Using immunohistochemistry expression of PR was observed in the nucleus and/or cytoplasm in the majority of human tumor specimens examined. Combinations of estrogen and progestins administered in vitro cooperate in promoting tumor secretion of vascular endothelial growth factor and, consequently, support tumor-associated angiogenesis. Further, dual treatment of bulk tumor cells with estrogen and progestin in vitro increased the numbers of subpopulations of putative tumor stem/progenitor cells enriched for cell surface CD133 and cytoplasmic aldehyde dehydrogenase-1 as determined by FACS (P<0.001). Concomitant treatment with the antiestrogen fulvestrant blocked this effect. Thus, ER- and/or PR-targeted therapies may hopefully offer new approaches to manage NSCLC in the clinic.

- (1) Weinberg O et al. Cancer Res 2005, 65:11287
- (2) Stabile L et al. Clin Cancer Res 2010, Epub ahead of print
- (3) Chlebowski R et al. J Natl Cancer Inst 2010, 102:1413
- (4) Chlebowski R et al. Lancet 2009, 374: 1243
- (5) Slatore C et al. J Clin Oncol 2010; 28: 1540
- (6) Mah V et al. Cancer Research 2007, 67:10484

Sources of Research Support: Early Detection Research Network NCI CA86366, the National Lung Cancer Partnership, the Specialized Programs of Research Excellence in Lung Cancer (SPORE) grant P50-CA90388, NCI and the DOD Lung Cancer Research Program. We are grateful to those patients who volunteered to provide clinical specimens for this work.

Nothing to Disclose: DCM-G, ELM, MA, H-WC, VM, LB, HJG, SH, DC, EBG, LG, RJP

Pub # P3-41

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Endocrine Neoplasia (1:30 PM-3:30 PM)

Title Chemoprevention of BBN-Induced Bladder Carcinogenesis by the Selective Estrogen Receptor Modulator Tamoxifen

Author String S Konnath George, V Tovar-Sepulveda, SS Shen, PM Dillard, SP Lerner, CL Smith
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Body Bladder cancer is the fourth most frequent tumor in men and ninth in women in the US. Most bladder carcinomas are a low grade papillary phenotype, and frequently are recurrent. Therapeutics able to prevent recurrence are a significant unmet need. Estrogen receptors (ERs) are ligand-regulated transcription factors which can promote cellular growth and survival, and treatment with the selective estrogen receptor modulator (SERM) tamoxifen, can block the growth of estrogen-dependent cells. ER β is the dominant ER expressed in bladder cancer cell lines and human bladder tumors. The goal of this study was to determine the impact of tamoxifen on bladder carcinogenesis induced by *N*-butyl-*N*-(4-hydroxybutyl) nitrosamine (BBN) supplied *ad libitum* in drinking water (0.05%) for 12 weeks to C57BL/6 female mice. Tamoxifen (~50[μ g/day) was administered via slow-release pellets either before (wk 5-8), during (wk 8-20), after (wk 20-32) or during+after (wk 8-32) BBN treatment (n=27-30/group). The study was terminated when mice were 32 weeks old. The mean bladder weights obtained from BBN only animals were significantly higher than control animals that did not receive BBN. This corresponded to a 76% incidence of urothelial cancer in the BBN only mice; controls were histologically normal. Treatment of mice with tamoxifen before or after BBN administration resulted in bladder weights that were not statistically different from the BBN only group; histopathological analyses for these two groups revealed cancer in 64% and 67% of before and after treatment groups, respectively. In contrast, mice receiving tamoxifen concurrent with BBN, or for the entire study period (wk 8-32) had bladder weights similar to control animals and only a low incidence of urothelial carcinoma (14% and 10%, respectively). Immunohistochemistry revealed that ER β expression was significantly reduced in mice that received tamoxifen concurrent with BBN treatment in comparison to BBN only animals. Surprisingly, ER α was detected in the bladders of all animals exposed to BBN, but was not found in any control mice. Like for ER β , ER α expression was lowest in mice that received tamoxifen concurrent with BBN treatment. These data indicate that tamoxifen administered concurrently with BBN prevents bladder carcinogenesis, and that this protective effect may be mediated by ER α and/or ER β . Administration of tamoxifen may be a therapeutic option for secondary prevention of human bladder cancer.

Nothing to Disclose: SKG, VT-S, SSS, PMD, SPL, CLS

Pub # P3-42
Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Endocrine Neoplasia (1:30 PM-3:30 PM)
Title VHL Disease: Implication of Age for Clinical Presentation and Follow-Up
Author String AM Vieites, G Sanso, G Levin, PLM Dahia, M Barontini
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Body von Hippel Lindau disease (VHL) is an inherited syndrome caused by mutations of the VHL gene. It predisposes to the development of retinal and CNS hemangiomas, renal or pancreatic cysts/tumors, endolymphatic sac tumors and pheochromocytomas (pheo). We evaluated both the clinical presentation and the outcome of a total of 49 patients, including 19 index cases (aged 4-21y), with VHL confirmed by molecular studies and their affected relatives detected by genetic screening (n=30). To characterize VHL gene mutations, a PCR-based SSCP strategy followed by direct sequencing was performed. All patients presented missense/nonsense mutations in the VHL gene. Patients were divided into 2 groups according to age: group 1, under 21y (n=31, 20males, 11females) and group 2, aged 24-75y (n=18, 11males, 7 females). The initial manifestation of VHL in group 1 was pheo in 25/31 patients (81%). During follow-up 20/25 pheo patients remained free of disease (median 6.5y, range:2-34y) while the other 5 patients presented hemangioblastoma of CNS(4), retina(2) and/or pancreatic tumor(1), with a disease-free interval of 4-23y (median 19y). Retinal hemangioblastoma (1), endolymphatic sac tumor(1) and CNS hemangioblastoma(4) were the initial presentation in the remaining 6 patients in this group. In 3/6 the tumor relapsed or another organ was involved within 1-2 years. In one case, pheo appeared after 16y of disease onset. Only one patient died. In group 2 the most common initial manifestation was CNS hemangioblastoma, observed in 8/18 patients (45%), followed by pheo in 6/18 patients (33%). The other clinical initial events were: retinal hemangioma in 2/18, clear cell renal carcinoma in 1/18 and neuroendocrine tumor of the pancreas in 1/18. Nine patients remained free of disease (follow up: 1-30y, median: 5y). Four patients died due to VHL, one of them post surgery and the others at 2, 5 and 11y of follow up. The mutations found in the index cases were: Arg167Thr(4), Arg161Term(3), Pro193Leu(2), Arg161Gln(1), Arg167Gln(1), Arg9His(1), Gly144Term(1), Leu163Arg(1), Pro138Arg(1), Pro86Ser(1), Thr124Ile(1), Val 84Leu(1), Arg200Pro(1). All detected mutations were associated with pheo, except Arg161Term. Our results confirmed that pheo is the predominant initial event in VHL in the pediatric group. They also show that these patients present a much better outcome than those with other tumors at disease onset. Since comorbid pathology can appear after a long disease-free period, lifelong surveillance is mandatory in both groups.

Sources of Research Support: Mincyt, Anpcyt grant PICT 25428 awarded to G.L.; CONICET grant PIP 5482 awarded to M.B.

Nothing to Disclose: AMV, GS, GL, PLMD, MB

Pub # P3-43

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Endocrine Neoplasia (1:30 PM-3:30 PM)

Title Coincidence or Connection: A Case of Papillary Thyroid Carcinoma in von Hippel-Lindau Disease

Author String AT Kraftson, JA Wyckoff
University of Michigan, Ann Arbor, MI

Body Von Hippel-Lindau (VHL) disease is a rare autosomal dominant genetic disease that causes germline inactivating mutations of the VHL suppressor gene. Consequently, affected individuals can develop neoplasms in various organs. Though there are many malignant manifestations, only one case of concurrent thyroid carcinoma has been reported in the literature [1]. We report an unusual case of previously undiagnosed VHL that initially presented with pheochromocytomas (PCC) and, years later, manifested with hypercalcemia metastatic pancreatic neuroendocrine carcinoma, spinal hemangioblastoma, and concurrent papillary thyroid carcinoma (PTC). The patient is a 35 year old woman who was found to have a right PCC and paraganglioma in 1991 and a left PCC in 1993, both treated with surgical intervention at our institution. The etiology of the PCC was not elucidated at the time. In 2009, she presented to an outside hospital with gastrointestinal complaints and imaging revealed pancreas, liver, and thyroid lesions. Fine needle aspiration of a thyroid nodule demonstrated papillary thyroid carcinoma. She returned to our institution for evaluation and management and was also found to have marked neck pain and hypercalcemia. Her pain necessitated hospitalization. Evaluation revealed PTHrP-mediated hypercalcemia. Liver biopsy was performed and demonstrated high grade neuroendocrine carcinoma. Review of the outside thyroid tissue slides confirmed PTC. The neck pain was evaluated with a spinal MRI and an intramedullary enhancing mass was identified. The patient's hypercalcemia was treated with intravenous fluids and bisphosphonate therapy. She underwent surgical removal of the spinal mass that was found to be a hemangioblastoma on histological analysis. Additionally, she underwent genetic counseling and subsequent testing was performed in a research lab of our institution. Exon 3 of the VHL gene was sequenced, the result was positive for a VHL mutation (R161Q), and the diagnosis of VHL was confirmed. This is the second reported case of VHL with concurrent papillary thyroid carcinoma. We review the literature and discuss possible connections between thyroid cancer and VHL.

(1) Reyes CV. Arch Intern Med. 1984. Feb; 144(2): 413

Nothing to Disclose: ATK, JAW

Pub # P3-44

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Endocrine Neoplasia (1:30 PM-3:30 PM)

Title Exocrine Pancreatic Tumors in Carney Complex: A Possible New Association Suggesting a Role for PRKAR1A in Pancreatic Oncogenesis

Author String S Gaujoux, F Tissier, B Ragazzon, V Rebours, E Saloustros, K Perlemoine, C Vincent-Dejean, J Le Borgne, I Cassagnau, X Bertagna, A Horvath, B Terris, A Carney, CA Stratakis, J Bertherat
Institut Cochin, INSERM U567, CNRS UMR8104, Paris, France; H[ocirc]pital Beaujon, AP-HP, Clichy, France; National Institutes of Health, Bethesda, MD; CHU H[ocirc]tel-Dieu, Nantes, France; CHU H[ocirc]tel-Dieu, Nantes, France; CHU H[ocirc]tel-Dieu, Nantes, France; Hopital Cochin, AP-HP, Paris, France; Mayo Clinic, Rochester, MN

Body Context: Carney complex (CNC) is a rare multiple neoplasia syndrome, inherited as an autosomal dominant trait, characterized by the development of endocrine tumors, lentiginosis and myxomas, and caused most frequently by inactivating mutations of the PRKAR1A gene.
Objectives: In our recent investigation of a large cohort of CNC patients, we identified several cases of pancreatic neoplasms. The aim of this study was to report this new association and PRKAR1A's possible involvement in pancreatic tumorigenesis.
Patients and Methods: Nine patients (2.5%) with CNC and pancreatic neoplasms in an international cohort of 354 CNC patients were identified; we analyzed the tumors from 6 cases. Immunohistochemistry (IHC) and PRKAR1A sequencing were done.
Results: Three men and 3 women with a mean age of 49 years had acinar cell carcinoma (n=2), adenocarcinoma (n=1) or intraductal pancreatic mucinous neoplasm (n=3). Five patients had a germline PRKAR1A mutation. In the two patients with acinar cell carcinoma, mutations were found in a homozygous state on the tumor, suggesting loss-of-heterozygosity (LOH). PRKAR1A expression was not detected in the neoplasms from CNC patients, whereas the protein was observed in sporadic pancreatic tumors i.e acinar cell carcinoma, pancreatic adenocarcinoma, intraductal pancreatic mucinous neoplasm and normal tissue.
Conclusion: An unexpectedly high prevalence of rare pancreatic tumors among CNC patients was found, especially acinar cell carcinomas, a rare form of pancreatic cancer, with less than 1 case per million of the United States population, and accounting for less than 1% of all pancreatic cancers. IHC and LOH studies suggest that PRKAR1A could function as a tumor suppressor gene in pancreatic tissue, at least in the context of CNC. Clinicians taking care of CNC patients should be aware of the possible association of CNC with a potentially aggressive pancreatic neoplasm.

Disclosures: JB: Investigator, HRA Pharma. Nothing to Disclose: SG, FT, BR, VR, ES, KP, CV-D, JLB, EC, XB, AH, BT, AC, CAS

Pub # P3-45

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Endocrine Neoplasia (1:30 PM-3:30 PM)

Title Exome Sequencing in Patients with Carney Triad Reveals Genetic Variations in Functionally Relevant Pathways

Author String AD Horvath, C Wassif, E Ball, M Azevedo, S Singh, J Epstein, J Niemela, E Saloustros, I Levy, JN Olivera, JA Carney, FD Porter, CA Stratakis
NIH, Bethesda, MD; NIH, Bethesda, MD; Mayo Clinic, Rochester, MN

Body Carney triad (CT) is a multi-tumor syndrome affecting the gastrointestinal tract, adrenals, paraganglionic system, lung, and esophagus. The disease was initially described in 1977 as an association of gastric epithelioid leiomyosarcoma [later renamed gastrointestinal stromal tumor (GIST)], extra-adrenal paraganglioma, and pulmonary chondroma. To date, the above three manifestations are known to be the most common ones, although in many cases only two out of the three tumors are seen. The disorder affects mostly young females; most known cases are sporadic and very few have been reported to cluster in families. Despite its apparent non-familial onset, a genetic etiology is clearly suggested by the concurrent appearance of three otherwise rare tumors.

We applied targeted exome enrichment (Agilent technologies) and subsequent massive parallel sequencing on SOLiD4 platform (Applied Biosystems) on four unrelated Carney triad patients presenting with all three most common tumors: GIST, lung chondroma and extra-adrenal paraganglioma. An average coverage of 40-fold per base was achieved, and filtering criteria to reduce false positive calls were applied. After filtering against existing SNP databases, and our own clinically unrelated next-generation sequencing datasets, between 509 and 908 novel sequence-altering variants per individual were identified, these including missense, nonsense, and splice changes (up to 3 bp from the junction). The variants were sorted according to their functional relevance, pathogenic potential and presence in the different patients, and a short list of gene-candidates was generated. Among the most favored candidates are genes involved in the tri-carbonic acid (TCA) cycle and cell respiration, cell signaling, and genes known to be have altered expression in related tumor conditions; experiments further confirming the selected genetic variants are ongoing.

Nothing to Disclose: ADH, CW, EB, MA, SS, JE, JN, ES, IL, JBO, JAC, FDP, CAS

Pub # P3-46

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Endocrine Neoplasia (1:30 PM-3:30 PM)

Title Haplotype Analysis Revealed Independent Events for an Identical Complex *TP53* Mutation in Two Unrelated Families with Diverse Cancer Profiles

Author String EM Pinto, RC Ribeiro, L Taja-Chayeb, LF Carrasco, GP Zambetti
St Jude Children's Research Hospital, Memphis, TN; St Jude Children's Research Hospital, Memphis, TN; St Jude Children's Research Hospital, Memphis, TN; Instituto Nacional de Cancerologia, Mexico City, Mexico; Hospital Escuela Servicio de Hematocologia Pediatrica, Tegucigalpa, Honduras; University of Tennessee College of Medicine, Memphis, TN

Body **Background:** Inherited germline mutations in the *TP53* gene are associated with an increased susceptibility to cancer. Namely, variability in tumor type and ages of onset within any family harboring the same germline *TP53* mutation can be quite extreme, precluding recognition of obvious genotype-phenotype associations. Here we report molecular findings in two different families with different core tumor components (adrenocortical tumor (ACT) in the first family; breast and sarcoma in the second) that harbor identical inherited germline complex *TP53* mutation. **Material and Methods:** *TP53* sequence and *TP53* haplotype analysis using SNPs and microsatellite markers for *TP53* locus were performed. DNA from blood and tumor samples in both families was also tested for the occurrence of Copy Number Variation (CNV) using Multiple Ligation-dependent Probe Amplification. **Results:** *TP53* sequence analysis reveals a germline, inherited mutation consisting of duplication of 7bp (c.324_330 dupl GGTTTCC) in exon 4 that generates a frameshift and a premature stop signal (position 150) in both families. Our data showed different haplotypes in each family ruling out the possibility of a Founder Effect. Loss of heterozygosity (LOH) with retention of mutated allele was observed in ACT and breast tumor. p53 immunostaining was observed only in ACT tissue. CNV for *TP53* locus was extensively observed in ACT patient and involved all *TP53* exons and was not observed in DNA from the patient's father who is a carrier of the same mutation, indicating a no inherited event. For sarcoma tumor tissue, CNV involved only *TP53* exons 1-5 and was not observed in breast tumor tissues which is carrier of the same genetic defect. **Conclusions:** The analysis of LOH, copy number variation and p53 immunostaining suggests that specific tissue factors interact with the p53 mutant and potentially account for the heterogeneity related to the tumor spectra in patients that harbor the same *TP53* mutation. Exciting questions could be addressed regarding the role of *TP53* mutation and CNV in cancer predisposition and also which of those changes are necessary and sufficient to cause neoplastic growth in a particular tissue, an issue that remains to be determined. The identification of new cases with identical mutations will permit the study of effects of environment, life style and other modifier genes on disease penetrance and spectrum of tumor types associated with identical genetic lesions.

Nothing to Disclose: EMP, RCR, LT-C, LFC, GPZ

Pub # P3-47

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Endocrine Neoplasia (1:30 PM-3:30 PM)

Title Wnt/beta-Catenin Regulates the T-Box Transcription Factor TBX1, the Candidate Gene of 22q11.2 Microdeletion/DiGeorge Syndrome, in Human Parathyroid Tumors

Author String S Corbetta, C Verdelli, V Vaira, M Meregalli, M Belicchi, A Terranegra, V Guarnieri, A Scillitani, L Vicentini, F Cetani, S Ferrero, M Frigerio, E Costa, B Ambrosi, Y Torrente, S Bosari, A Spada
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Body Wnt/beta-catenin pathway has been shown to be upregulated in parathyroid carcinomas due to *CDC73/HRPT* gene inactivating mutations and loss of parafibromin and APC. Wnt/beta-catenin signalling has been identified as a crucial upstream regulator of *TBX1* gene expression in a mouse model of DiGeorge syndrome (DGS). We tested the hypothesis that the embryonic transcription factor TBX1, involved in cell differentiation during parathyroid organogenesis, might be a target of the beta-catenin accumulation in parathyroid cancers. By Real-Time PCR, western blot and immunohistochemistry, we demonstrated that TBX1 mRNA and protein were expressed at high levels in 23 typical parathyroid adenomas showing a clearly positive staining in parathyroid PTH+ cells with a cytoplasmic and nuclear localization. A similar expression pattern could be detected in parafibromin-expressing parathyroid cancers (n=3). By contrast, TBX1 mRNA and protein were definitely downregulated in parafibromin negative parathyroid carcinomas (n=14) and in 3 metastatic lesions, suggesting that Wnt/beta-catenin activation might inhibit TBX1 expression. Functional studies were performed in HEK293 cells, since they expressed TBX1 mRNA and protein. In HEK293 cells TBX1 mRNA and protein were inhibited by beta-catenin accumulation induced by 24-hours treatment with 1-20 nM lithium chloride, while beta-catenin degradation induced by pretreatment with 10-100 nM calyculin for 40 min determined an increase in TBX1 expression levels. Moreover, the activation of the calcium sensing receptor (CaSR) by stimulating HEK293 cells stably transfected with the human CaSR, with increasing calcium concentrations as well as with increasing concentrations of the potent and selective CaSR agonist R-568, induced a significant reduction in TBX1 mRNA levels after 24 hours incubation. Nonetheless, in human adult normal parathyroid tissues (n=3), TBX1 protein was expressed in endothelial CD31+, alpha-SMA+ cells while parathyroid chief cells were negative. During embryonic development impaired *TBX1* gene dosage, both deletion and duplication, is associated to DGS phenotype: further studies will investigate the effect of the beta-catenin accumulation induced inhibition on TBX1 expression in parathyroid cancers. In conclusion, the present study: 1) identified a new target of the Wnt/beta-catenin pathways in parathyroid tumors and 2) suggested that TBX1 impaired expression contributes to parathyroid tumorigenesis.

Nothing to Disclose: SC, CV, VV, MM, MB, AT, VG, AS, LV, FC, SF, MF, EC, BA, YT, SB, AS

Pub # P3-48

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Endocrine Neoplasia (1:30 PM-3:30 PM)

Title Correlation of Serum Ionized Calcium, Parathyroid Tumor Weight, and Positive Sestamibi Scan in Patients with Primary Hyperparathyroidism

Author String TD Hoang, VQ Mai, PW Clyde, MC Royer, KM Shakir
National Naval Medical Center, Bethesda, MD

Body **Background:** Presently there are no biochemical markers to predict the probability of a positive Tc-99m sestamibi scan in patients with primary hyperparathyroidism. We studied the relationship between various biochemical parameters and tumor size in patients with positive and negative Tc-99m sestamibi scans. **Method:** Retrospective review of data for 59 patients with primary hyperparathyroidism was performed. 31 patients had positive sestamibi and 28 had negative sestamibi scans. Serum total calcium, ionized calcium, intact PTH, albumin, alkaline phosphatase, phosphorus, 25-hydroxy vitamin D, 1,25-hydroxy vitamin D, 24-hour urine calcium levels and parathyroid tumor weight were analyzed. **Results:** The average values of serum total calcium, phosphorus and PTH were 11.21 ± 0.46 mg/dL, 3.07 ± 0.57 mg/dL, and 121.60 ± 46.52 pg/mL, respectively, for the positive-sestamibi group and 10.98 ± 0.49 mg/dL, 3.19 ± 0.57 mg/dL, and 125.01 ± 61.03 pg/mL, respectively, for the negative-sestamibi group. However, the values of these two groups were not statistically different. Similarly, other biochemical parameters were not statistically different between the two groups, except for ionized calcium level and tumor weight. The ionized calcium level was 6.23 ± 0.34 mg/dL for the positive-sestamibi group and 6.03 ± 0.27 mg/dL for the negative-sestamibi group ($p = 0.014$). The tumor weight was 848.4 ± 630.2 mg for the positive-sestamibi group and 239.0 ± 160.00 mg for the negative-sestamibi group ($p = 0.006$). Using a cut-off level of 6.2 mg/dL for ionized calcium, 70% of the patients had a positive sestamibi scan. Using a cut-off level of 6.3 mg/dL, 78% of the patients had a positive sestamibi scan. **Conclusion:** In primary hyperparathyroidism, there was no correlation between the levels of serum total calcium, intact PTH, and the positivity of Tc-99m sestamibi scan. According to our results, a serum ionized calcium level of at least 6.2 mg/dL will predict approximately 70% probability of a positive sestamibi scan. Those with positive sestamibi scans were found to have higher parathyroid tumor weight compared to those with negative sestamibi scans. Further studies with larger patient populations are needed.

Nothing to Disclose: TDH, VQM, PWC, MCR, KMMS

Pub # P3-49

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Endocrine Neoplasia (1:30 PM-3:30 PM)

Title A Case of Metastatic Struma Ovarii: The Importance of Close Follow-Up

Author String RC Schulman, AB Schultz
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Body

Background:
Struma ovarii is a rare ovarian teratoma consisting of greater than 50% thyroid tissue and comprises 1% of all ovarian tumors and 3% of ovarian teratomas. 5% of cases exhibit hyperthyroidism. Malignant change, as defined by histologic criteria used in thyroid carcinoma, can occur, but metastatic spread is rare and occurs in only 5% of cases (1). We present a case of struma ovarii with metastatic spread.

Case:
A 19 year-old female was found to have abdominal distention on a routine physical exam in 2006. A CT scan with contrast showed a 22 cm left sided ovarian mass and pelvic ascites. Laboratory values included: TSH 0.02 (0.34-4.82), FT3 5.3 (2.3-4.2), FT4 0.50 (0.61-1.76), anti-thyroglobulin and anti-TPO antibodies and TSI negative. Thyroid ultrasound showed a normal appearing gland without nodules. The patient underwent a unilateral salpingo-oophorectomy and omentectomy. Pathology was consistent with struma ovarii with extensive necrosis, noting that it was impossible to rule out a well-differentiated follicular carcinoma. Pelvic washing and omental nodes were without malignant cells. Post-operatively, the TSH was in the normal range and a CT scan showed no evidence of tumor or metastatic deposits. A CT scan from 2008 and unchanged in 2009 showed 3 new pulmonary nodules and 4 well-circumscribed liver masses. In November of 2009 and repeated in May of 2010, the patient was found with a below normal TSH, 0.114 and 0.277 (0.450-4.500) respectively. The patient was referred for an endocrine evaluation. A thyroglobulin level was found to be significantly elevated at 15,973 ng/ml (anti-thyroglobulin antibody negative). WBI scan showed uptake in the thyroid, chest and abdomen. The patient underwent surgical resection of a peritoneal mass and pathology was consistent with peritoneal strumosis with foci of papillary thyroid carcinoma, follicular variant. A diagnosis of metastatic struma ovarii was made. The planned treatment for this patient includes total thyroidectomy, RAI ablation, TSH suppression, serial thyroglobulin and WBI scans, with further RAI treatment as needed.

Conclusion:
Metastatic spread is a rare but serious complication of struma ovarii. This case illustrates the importance of close follow-up for patients with struma ovarii, especially if the initial pathology is not definitively benign. Serial thyroglobulin levels or closer attention to CT scan changes would have likely identified metastatic struma ovarii earlier in this case.

(1) Roth LM et al., Pathology 2007; 39: 139.

Nothing to Disclose: RCS, ABS

Pub # P3-50

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Endocrine Neoplasia (1:30 PM-3:30 PM)

Title Metastatic Papillary Thyroid Carcinoma Discovered in Struma Testis

Author String S Barakat, JL Odem, U Khan
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Body

Introduction:

Identifying thyroid tissue in an ovarian teratoma or Struma Ovarii (SO) is not uncommon. Several cases of thyroid carcinoma in SO have been reported. However, identification of thyroid tissue in a testicular teratoma (Struma Testis) is very rare. We report a rare case of papillary thyroid carcinoma (PTC) in a testicular teratoma.

Case Report:

A 56 year-old Caucasian male an inguinal hernia, and undescended left testicle, was admitted with right lower quadrant abdominal pain due to appendicitis. He underwent a laparoscopic appendectomy, and left orchietomy. There was no history of smoking, exposure to radiation, or a family history of thyroid cancer. The testicle measured 4.5 x 2.6 x 2.0 cm and weighed 12 g. Sectioning showed a solid well circumscribed yellow lesion measuring 1.6 x 1.6 x 1.5 cm. Microscopic examination showed a monodermal teratoma composed of thyroid parenchyma with PTC, predominantly follicular growth pattern. A CT scan of the chest with intravenous contrast showed a well circumscribed soft tissue right hilar mass measuring 2.2 x 2.4 x 2.7 cm with multiple small pulmonary nodules within the lower lung fields bilaterally. The right hilar mass showed mild FDG activity on FDG-PET fusion scan. A wedge biopsy of this nodule showed PTC. Thyroid ultrasound showed a normal sized, homogeneous gland, without nodules, calcifications, or abnormal vascularity. A total thyroidectomy was performed. Final pathology showed a normal thyroid gland with one microscopic focus (0.5 mm) of PTC with follicular architecture. A whole body scan using 1.5 mCi of I-123 showed residual uptake in the neck is 2.5%. He was treated with 200 mCi of I-131. A post therapy WBS did not show any new areas of increased uptake. A non-stimulated thyroglobulin <0.2 ng/ml, and TgAb <0.9.

Discussion:

A testicular teratoma with thyroid tissue seems to be first described by Ewing in his book [ldquo]Neoplastic Disease[rdquo] in 1919. Several references either commented briefly that this entity is very rare or did not even mention it. In 1975 the first report for a malignant struma of the testis with metastasis to the para-aortic lymph nodes was published in the Indian Journal of Cancer. Another case of a pleomorphic atypical thyroid adenoma in strum testis was reported in 1982. Our case is the first case of metastatic papillary thyroid carcinoma that was discovered in a Struma Testis as well as the micro PTC. What should be considered the primary tumor is debatable.

Nothing to Disclose: SB, JLO, UK

Pub # P3-51

Session Information POSTER SESSION: CLINICAL - Endocrine Tumor Case Reports (1:30 PM-3:30 PM)

Title Unusual Clinical Presentation of Intrathyroidal Parathyroid Carcinoma

Author String MTA Reis, BB Mendonca, MR Menezes, V Dalalio, FLM Montenegro, CHA Teixeira, AAFdO Hoff, PHS Correa, RM Martin
Clinics Hospital of Faculty of Medicine of São Paulo University, São Paulo, Brazil; Cancer Institute of São Paulo, São Paulo, Brazil; Private Practice, Uberaba, Brazil; Clinics Hospital of Faculty of Medicine of São Paulo University, São Paulo, Brazil; Cancer Institute of São Paulo, São Paulo, Brazil; Cancer Institute of São Paulo, São Paulo, Brazil

Body **Background:** Parathyroid cancer (PC) is an extremely aggressive malignancy and accounts for <1% of cases of primary hyperparathyroidism (pHPT). **Clinical case:** Three years ago, a 44-yr-old woman sought medical attention for a cervical mass. Cervical ultrasound detected a thyroidal nodule (2.4x1.4x1.2cm) whose FNAB showed no follicular or papillary differentiation and a partial thyroidectomy (TD) was performed. The presence of thick fibrous bands, mitotic activity, vascular and capsular invasion at histopathological examination and positive immunohistochemistry for PTH revealed that the nodule was, in fact, an intrathyroidal parathyroid carcinoma. For the first time, calcium and PTH levels were measured (8.7mg/dL, NV=8.8-10.2mg/dL; 54pg/mL, NV=11-62pg/mL) without evidence of skeletal or renal disease related to pHPT. Afterwards, she underwent 3 surgeries to excise loco-regional PC metastasis although sestamibi scintigraphy was negative. Two years after first surgery, for the first time, she displayed hypercalcemia (10.8mg/dL) with high PTH levels (221pg/mL), which became higher (Ca 13.2mg/dL; PTH 343pg/mL), concomitantly thorax CT scan showed pulmonary micronodules. In order to maintain hypercalcemia below 13mg/dL (oligosymptomatic patient), she received bimonthly bisphosphonate infusion (3 doses), as she presented increase in bilateral pulmonary lesions and PTH levels around 1000pg/mL but had no evidence of cervical disease. To improve her quality of life, cryoablation of the biggest thoracic nodules was performed instead of bilateral thoracotomy. Few days later, PTH decreased from 1519 to 240pg/mL and she presented hungry bone syndrome. Noteworthy, she had renal cysts and her sister was diagnosed as having pHPT, which suggest HPT-jaw tumor syndrome, a disorder caused by mutations in the *HRPT2* gene where the incidence of PC is elevated. Both sisters did not have jaw tumor. **Conclusion:** The diagnosis and appropriate treatment of PC are particularly difficult. Usually, patients are diagnosed due to a large neck mass, high PTH levels and signs of severe hypercalcemia with bone disease and renal manifestations. In this case, pHPT was not initially considered because of the intrathyroidal parathyroid topography however immunohistological analyses facilitated PC diagnosis and allowed the patient to be kept under close surveillance. Surprisingly, she developed hypercalcemia only 2 years after diagnosis, when she presented pulmonary metastasis.

Nothing to Disclose: MTAR, BBM, MRM, VD, FLMM, CHAT, AAFdOH, PHSC, RMM

Pub # P3-52

Session Information POSTER SESSION: CLINICAL - Endocrine Tumor Case Reports (1:30 PM-3:30 PM)

Title Failure of Multiple Imaging Modalities to Identify Metastatic Tumor Location in Recurrent Parathyroid Carcinoma

Author String J Maaske, MH Pisaneschi, P Raghu, A Babu
John H Stroger, Jr Hospital of Cook County, Chicago, IL

Body Parathyroid Carcinoma is a rare cause of primary hyperparathyroidism. Patients presenting with the constellation of markedly elevated parathyroid hormone(PTH) and calcium(Ca) and a palpable neck mass raise its diagnostic consideration. Primary surgery is the best chance for cure in patients, as recurrence is common. Multiple modalities can be undertaken to attempt tumor recurrence localization, though these do not always yield positive results.

A 43 year old male diagnosed with parathyroid carcinoma in 2003 requiring two neck explorations. Recurrence occurred one year later and he underwent extensive neck dissection with negative postoperative sestamibi and MRI at other institutions. In 2009 he presented to our institution with an incidentally noted Ca of 16.1mg/dl (normal 8.5-10.5mg/dl) and PTH of 1116pg/ml (normal 6-65pg/ml). Full body sestamibi, CT neck and PET failed to reveal the metastatic tumor location. CT chest demonstrated a 17x17mm mass in the upper mediastinum. He underwent sternotomy with no postoperative change in calcium and reactive lymph node on pathology. Serum calcium and PTH levels continued to climb, up to 22mg/dl and 1700pg/ml, respectively. He became refractory to IV bisphosphonate therapy, with effectiveness lasting less than two weeks. He was unable to tolerate cinacalcet due to side effects and a trial of chemotherapeutic agents failed to improve symptoms or calcium. He continued to clinically deteriorate and underwent invasive selective venous sampling which did not demonstrate a regional gradient. Repeat CT chest demonstrated an interval increase in size (26x17mm) of the previously seen (17x17mm) upper mediastinal mass. He underwent repeat sternotomy and distal tracheal resection, with postoperative normalization of calcium and PTH decline to 100pg/ml. He is maintained on oral calcium and vitamin D.

Initial surgery offers the best chance at cure for parathyroid carcinoma. With recurrence, there are several modalities available to localize tumor burden. Despite this, there are some circumstances in which radiologic imaging does not correlate with markedly elevated tumor markers. This case is unique in that the chest metastatic disease was not positive on sestamibi, which has a sensitivity of 86-90%, or on PET scan. In this case, a multi-disciplinary approach to patient care should be undertaken in order to provide symptomatic relief and to continue to diligently investigate for the source of metastasis.

Nothing to Disclose: JM, MHP, PR, AB

Pub # P3-53

Session Information POSTER SESSION: CLINICAL - Endocrine Tumor Case Reports (1:30 PM-3:30 PM)

Title Parathyroid Cancer: Sorafenib May Be an Option for Treatment

Author String G Etzrodt-Walter, S Walter, H Etzrodt
Endokrinologie-Ulm, Ulm, Germany; Medical Clinic University Ulm, Ulm, Germany

Body

Background:
Primary hyperparathyroidism is a common disorder. Parathyroid cancer is uncommon and adjuvant chemotherapy has not yet proven effective. We present a case in which therapy with cinacalcet was poorly tolerated, but therapy with sorafenib resulted in a decline of serum calcium levels and stable tumor disease.

Case:
A 38 year old woman presented in September 2004 at her dentist with an inflammatory mass in the molar region on her right upper jaw. Histology revealed [ldquo]giant cell granuloma[rdquo]. In laboratory testing detection of hypercalcemia and primary hyperparathyroidism. A multiple endocrine neoplasia typ I and II where excluded. Surgery was performed without complications and normalization of serum-Calcium. Histology showed no cysts and no signs of malignancy.

Two years later again calcium levels rose. Metastatic disease was found in the follow up a second parathyroidal adenoma and multiple lung metastasis. After an expanded surgery including sternotomy a palliative situation was found. Treatment with cinacalcet was started, well tolerated. The calcium levels remained elevated and did not change with the addition of zoledronic acid.

The following 2 years the disease was stable but in 2010 again calcium levels rose. A jugular mass developed. Cinacalcet was no longer tolerated at a dosis of 90 mg/d. After the 4th surgery a treatment with sorafenib was started and is actually very well tolerated. Calcium levels at 1.9 mmol/l ionized Ca. and pulmonary metastasis are stable.

Conclusion:
In parathyroid cancer sorafenib may be an therapeutic option.

Nothing to Disclose: GE-W, SW, HE

Pub # P3-54

Session Information POSTER SESSION: CLINICAL - Endocrine Tumor Case Reports (1:30 PM-3:30 PM)

Title Why Do My Bones Hurt?

Author String AK Chunduri, C Valluri, SJ Sahota, D Ciltea, N Haller, A Powell
Akron General Medical Center, Akron, OH

Body

Introduction
Parathyroid carcinoma is a rare condition with an incidence of less than 1% in the United States and Europe. We report an unusual case of Parathyroid carcinoma in a patient who presented for routine office visit.

Case report
A 55-year-old Caucasian female came to the clinic for routine visit. On review of systems, the patient complained of fatigue, bone pain, intermittent constipation and myalgias. Initial labs revealed an elevated serum calcium level of 13.3mg/dl. The patient underwent further workup for hypercalcemia and her intact PTH level was found to be significantly elevated at 527pg/ml. U/S of the neck showed a hypo echoic area at the right inferior parathyroid region. Based on the location, the patient was suspected of having a parathyroid adenoma and underwent a right inferior parathyroidectomy. Pathological specimens were positive for parathyroid carcinoma with positive margins, and the patient had repeat neck surgery with En bloc resection. Intraoperative PTH monitoring showed improvement. The Oncologist did not recommend chemotherapy. The patient is currently following with her Endocrinologist every six months and has stable PTH and calcium levels.

Discussion
Parathyroid carcinoma is a rare condition seen in up to 5% of patients with primary hyperparathyroidism. Parathyroid carcinoma may be familial or sporadic. The familial form may be associated with familial hyperparathyroidism and/or hereditary hyperparathyroid jaw tumor syndrome. The exact etiology for parathyroid carcinoma is unclear, but is believed to be related to radiation or gene mutations, including HRPT2. Current treatment of choice includes surgical, En bloc resection, medical therapy, and chemotherapy. Parathyroid carcinoma has the potential to metastasize, but can be managed medically by controlling hypercalcemia if discovered at an early stage. Genetic testing is available, and recommended, for the HRPT 2 gene mutation.

Conclusion
Early detection and treatment will prevent the morbidity and mortality associated with hypercalcemia.

Nothing to Disclose: AKC, CV, SJS, DC, NH, AP

Pub # P3-55

Session Information POSTER SESSION: CLINICAL - Endocrine Tumor Case Reports (1:30 PM-3:30 PM)

Title Central Diabetes Insipidus and Pituitary Hypofunction Associated with Acute Myeloid Leukemia

Author String S Narayana, P Otterbeck, K Soe, A Gliwa, SI McFarlane
State University of New York- Downstate Medical Center, Brooklyn, NY

Body

Introduction:
Central diabetes insipidus (CDI) and pituitary hypofunction have been rarely reported in acute myeloid leukemia (AML). We present a case of AML with CDI and pituitary hypofunction without any discernible hypothalamic or pituitary lesions.

Clinical case:
This is a 50 year old man with hypertension and dyslipidemia who was hospitalized initially for poor appetite, weight loss, lethargy, polydipsia and polyuria (4.5L/day). His labs were significant for pancytopenia and hypernatremia of 158 mEq/L, $n < 145$. Further assessment with bone marrow biopsy, immunophenotype and karyotype (45 XY, t (3:3), -7) confirmed the diagnosis of AML with monosomy 7. He was started on chemotherapy with cytarabine and idarubicin. He also had DDAVP challenge test to r/o DI. Patient responded to the test dose confirming the diagnosis of Central DI with reduction of urine volume from 4.5 L/day to 1.9L/day. Urine osmolality increased from 94 to 553 mosm/L and serum Na decreased from 158 to 146 mEq/L. Additional work up revealed central hypothyroidism (TSH-0.65 mIU/ml (0.35-5.50), Free T4 - 0.58 ng/dl (0.89-1.76)) and central hypogonadism (LH- 2.65 mIU/ml, (1.5-9.3), FSH- 1.54 mIU/ml, (1.4-18.1), Total Testosterone- 191 ng/dl, (241-827), SHBG- 40 nmol/L, (18-47) with normal cortisol and ACTH levels. MRI of the pituitary did not reveal any abnormalities. The patient was treated with intranasal desmopressin 10 mcg one time two times a day and Levothyroxine 88mcg daily. He responded well with resolution of polyuria and hypernatremia. Since patients with AML and monosomy 7 (as in our patient) generally have poor prognosis, our patient who indeed failed two attempts at chemotherapy, opted for home hospice care with continued DDAVP therapy.

Conclusion:
AML can rarely present as central DI, with only few case reports to-date. It can also be associated with panhypopituitarism or selective deficiency of pituitary hormones as in our patient. While it is tempting to assume pituitary infiltration with leukemic cells as a plausible mechanism, MRI in our patient did not support such assumption. Physicians should be aware of the association of AML with central DI and Pituitary deficiencies in order to provide timely interventions for such severely ill patients.

Nothing to Disclose: SN, PO, KS, AG, SIM

Pub # P3-56

Session Information POSTER SESSION: CLINICAL - Endocrine Tumor Case Reports (1:30 PM-3:30 PM)

Title Virilization and Hypertension in a Perimenopausal African-American Female Resolved after Oophorectomy: Case Report of Ovarian Leydig Cell Tumor

Author String SD Corathers, M Salehi
University of Cincinnati, Cincinnati, OH; Cincinnati Children's Hospital Medical Center, Cincinnati, OH

Body
Introduction: Hyperandrogenemia and oligomenorrhea are often a result of Polycystic Ovarian Syndrome, however this is a diagnosis of exclusion. We present a case of rapidly progressing virilization with history of chronic mild hirsutism, in which ultrasound identified right adnexal cysts, however the ultimate diagnosis was Leydig cell tumor on the contralateral ovary.
Clinical Case: A 45-year-old African American woman was evaluated for worsening hirsutism (daily facial shaving), oligomenorrhea, voice deepening, acne and irritable mood over 1-2 years. Menarche was at age 12 with regular menstrual cycles. She became pregnant twice without difficulty in her 20s. In her 30s, she gained weight, developed hirsutism with irregular menstrual cyclicality. Also, she developed hypertension that required hospitalization for hypertensive emergency, which resolved with better blood pressure control (taking up to four antihypertensive medications). A right ovarian cyst, found incidentally, was removed three years ago. On exam, she was obese (BMI 35kg/m²), virilized (1.8cm clitoromegaly), and hypertensive (BP 156/95mmHg). Fasting laboratory evaluation showed elevated total and free testosterone (592 and 17.54ng/dL, respectively), low LH and FSH (9.6 & 5.4mIU/mL, respectively), mildly elevated prolactin (24.7ng/mL), and normal DHEA, DHEA-s, 17-OH-Progesterone, thyroid function, 24 hour urine cortisol, glucose, and insulin. Pelvic ultrasound demonstrated cystic lesions in the right adnexa. Bilateral oophorectomy revealed a 1.8cm benign Leydig Cell Tumor of the left ovary. Six weeks post-operatively, patient felt significantly better regarding mood and quality of voice; shaving frequency decreased to 3x/week (total testosterone of 10ng/dL). Blood pressure was 128/78 with a single agent.
Clinical Lessons: Leydig cell tumors are rare ovarian tumors that are usually unilateral and benign. Frequently, small size makes ultrasound unreliable, as was the case for our patient. MRI is more sensitive for identifying these lipid rich tumors, but definitive diagnosis is based on histology. In our patient, testosterone levels fell dramatically after oophorectomy and symptoms improved immediately. This case highlights that clinical manifestations of virilization, regardless of longstanding mild hirsutism or negative ultrasound imaging should prompt further evaluation. Accurate diagnosis resulted in identification of a curable source of androgen excess for this patient.

Nothing to Disclose: SDC, MS

Pub # P3-57

Session Information POSTER SESSION: CLINICAL - Endocrine Tumor Case Reports (1:30 PM-3:30 PM)

Title A Case of Long-Standing Painful Skin Rash and Depression

Author String P Sherchan, D Gang, CP Granada, Y Altoraiweb, S Sen
Baystate Medical Center, Springfield, MA

Body

Case: A 42 YO female presented with depression, 40 lb weight loss, diarrhea and progressively worsening maculopapular, migratory, desquamative, painful rash involving the extremities, trunk, and perineum for over 6 months, resistant to standard therapy. Biopsy showed spongiotic dermatitis with focal keratinocytes vacuolization and parakeratosis, features consistent with necrolytic migratory erythema (NME). Subsequently drawn serum Glucagon level was 627 (40-130 pg/ml). She remained euglycemic with mean fasting blood glucose of 80 mg% and concomitant fasting insulin level of 17 [micro]u/ml. HbA1C was 5.2%. CT demonstrated a 3.4[times]2.7[times]2.5 cm mass in the tail of pancreas. MRI and octreotide scan confirmed the lesion with no evidence of metastatic disease. A diagnosis of glucagonoma associated with characteristic necrolytic migratory erythema (NME) rash was made. She underwent distal pancreatectomy which revealed a well differentiated pancreatic neuroendocrine tumor with (+) immunohistochemical staining for glucagon. Within 1 day of tumor resection, there was resolution of NME rash and near complete resolution in 2 weeks. Postoperatively, her glucagon level decreased to 37 pg/ml and insulin level was 4.1 [micro]u/ml with synchronous fasting blood glucose of 181. Of note, her blood glucose levels remained satisfactory pre-operatively in spite of high glucagon levels and began to rise only after partial pancreatectomy with subsequent stabilization at impaired glucose tolerance level.

Discussion: Reports of diabetes in glucagonoma vary from 38 to 94% but we did not find hyperglycemia in our case. We entertained the possibility of a compensatory mechanism of beta cell hyperplasia that was counteracting the high glucagon levels. After detailed pathological evaluation of pancreatic tissue from normal, insulinoma and glucagonoma (our case) patients, we found no evidence of the same by immunohistochemical staining.

The characteristic skin lesion of NME, has been described in upto 70% of patients with glucagonoma. In our patient, rapid post-operative resolution of both the rash and depression are indicative of significant paracrine properties of glucagon in skin and CNS.

Conclusions: The strength of manifestation of hyperglucagonemia seems widely variable depending on the organ system involved. The skin manifestation seemed out of proportion to the level of hyperglucagonemia whereas no effect on carbohydrate metabolism and compensatory beta cell hyperplasia were noted.

Nothing to Disclose: PS, DG, CPG, YA, SS

Pub # P3-58

Session Information POSTER SESSION: CLINICAL - Endocrine Tumor Case Reports (1:30 PM-3:30 PM)

Title The Diverse Spectrum of Non-Islet Cell Tumor-Induced Hypoglycemia (NICTH) -- Three Case Reports

Author String K Kumareswaran, S Curran, M Thomas, R Kirolos, S Jefferies, A Melvin, G Wark, P Sonksen, D Russell-Jones, P Corrie, D Halsall, H Simpson
Addenbrooke's Hospital, Cambridge, UK; Bedford Hospital, Bedford, UK; Royal Surrey County Hospital, Guildford, UK; St Thomas' Hospital London, London, UK

Body

Background:
NICTH is a rare cause of hypoglycaemia, attributed to overproduction of big insulin-like growth factor II (pro IGFII), resulting in insulin-like effects and negative feedback on GH secretion. It typically presents in the setting of large mesenchymal or epithelial tumours. Raised IGFII:IGFI, and low plasma insulin and C-peptide in the context of hypoglycaemia suggest the diagnosis. Reduction of tumour burden is the definitive treatment. High dose glucocorticoids and/or recombinant human growth hormone (rhGH) are effective medical treatment options.

Clinical cases:
A 61 year old man, with a 19-year history of meningial haemangiopericytoma, presented with hypoglycaemia requiring continuous dextrose infusion. Investigations revealed suppressed insulin (<2pmol/l, n 0-60 pmol/l), low C-peptide (41pmol/l, NR 174-960pmol/l), raised IGFII:IGFI (13.3, NR<10). Liver biopsy confirmed metastatic haemangiopericytoma. Advanced tumour stage precluded surgery. Dexamethasone 3mg, rhGH 1.4mg, and corn starch were commenced, with subsequent resolution of symptomatic hypoglycaemia facilitating discharge home, reduction in IGFII:IGFI (6.3), and increase in HbA1c (4.4% to 4.9%).
A previously well 42 year old lady presented unconscious with glucose of 1.9mmol/l. USS revealed a retroperitoneal mass. Haemangiopericytoma was diagnosed on laparotomy, with subsequent resolution of hypoglycaemia. Preoperatively, free IGFI and IGFII were elevated and IGFBP3 was suppressed; these normalised following surgery. Further hypoglycaemia associated with tumour recurrence was effectively managed with repeated resections and chemotherapy.
A 78 year old lady presented with seizures and hypoglycaemia. Plasma glucose 1.8mmol/l was recorded during 72-hour fast, with concurrent insulin <2pmol/l and C-peptide 174pmol/l, excluding insulinoma. Elevated IGFII:IGFI (11.5) confirmed NICTH. CT chest/abdomen/pelvis, Octreotide and MIBG scans failed to localise a tumour. Hypoglycaemic episodes were successfully controlled with diet and regular cornstarch.

Conclusion:
We describe 3 diverse cases of NICTH which highlight the challenges faced in managing this rare syndrome. The use of high dose Dexamethasone and rhGH in the first case enabled a symptom-free period for 6 months. In comparison, repeated tumour resection controlled the hypoglycaemia in the second case for 18yrs. No tumour source was identified in the third case, but hypoglycaemia was effectively managed with simple measures.

Nothing to Disclose: KK, SC, MT, RK, SJ, AM, GW, PS, DR-J, PC, DH, HS

Pub # P3-59

Session Information POSTER SESSION: CLINICAL - Endocrine Tumor Case Reports (1:30 PM-3:30 PM)

Title Olfactory Neuroblastoma as a Cause of Severe Ectopic Cushing Syndrome

Author String JY Han, M Yeh, AP Heaney
David Geffen School of Medicine at UCLA, Los Angeles, CA

Body Olfactory neuroblastoma is a rare cause of ectopic ACTH secretion and Cushing's Syndrome and the diagnosis is often challenging. We describe a 59-year-old male who initially presented to an outside facility with hypokalemia, hypertension, hyperglycemia, and poorly differentiated mandibular carcinoma with endocrine features. He endorsed a 1 year history of nasal congestion, epiphora and right periorbital edema. He was treated with 2 cycles of chemotherapy (Carboplatin and Etoposide) with an objective tumor response. Two months later, he developed acute onset abdominal pain with CT evidence of a perforated diverticulum. Chemotherapy was halted and hemicolectomy of the sigmoid colon with colostomy placement was undertaken. Bilateral adrenal hyperplasia was also noted on CT and a work-up for Cushing's Syndrome was initiated. Plasma ACTH (500pg/mL) and cortisol (60ug/dl) were elevated, a diagnosis of ACTH-dependent Cushing's Syndrome was made and he was started on ketoconazole, insulin, and potassium supplements. One month later, he was transferred to UCLA with ongoing symptoms of severe Cushing's Syndrome with hypokalemia and poor wound healing. CT/ PET imaging revealed an enlarged FDG-avid right submandibular gland and several enhancing enlarged lymph nodes with complete paranasal sinus opacification. Pituitary MR showed no abnormalities. 24hour urine cortisol was elevated at 553ug/d and serum cortisol and ACTH levels were 30mcg/dL and 223pg/mL respectively despite ketoconazole 400mg and Octreotide 200mcg TID. Resection of the right skull-based mass revealed an olfactory neuroblastoma which was immunopositive for synaptophysin, chromogranin, S-100 and weak-negative for ACTH. We elected to perform urgent bilateral adrenalectomy due to his persistent Cushing's Syndrome despite maximal medical therapy. His hospital course was further complicated by additional intestinal perforation, poor wound healing and nephrogenic diabetes insipidus secondary to severe hypokalemia. Olfactory neuroblastoma is a rare neoplasm representing 3-10% of sinonasal malignancies and an extremely rare cause of ectopic Cushing's Syndrome. Nonspecific symptoms of nasal obstruction, epiphora and epistaxis make diagnosis difficult. Surgical resection is the treatment of choice; other options include neoadjuvant radiation therapy and chemotherapy. However, the 5 year survival rate is poor at 45% although ACTH production imparts a more favorable prognosis due to earlier clinical presentation.

Nothing to Disclose: JYH, MY, APH

Pub # P3-60
Session Information POSTER SESSION: CLINICAL - Endocrine Tumor Case Reports (1:30 PM-3:30 PM)
Title False-Positive ACTH Caused by Heterophil Antibody in an Obese Patient Considered for Possible Cushing Syndrome
Author String E Melcescu, KK Ng, WA Salameh, CA Koch
University of Mississippi Medical Center, Jackson, MS; Mayo Clinic, Rochester, MN; Quest Diagnostics, Sar Juan Capistrano, CA

Body Background: A number of factors could influence the accuracy of immunoassay evaluations: preanalytical, analyte-independent and -dependent. Of these, heterophilic antibodies (HA) are particularly troublesome because they are difficult to detect and prevent. The analytical interference caused by HA can lead to erroneous treatment decisions and increased cost. Studies have shown that 30-40% of the population have HA (1), which result in an interference rate of 0.05% to [ge]6% in commercial immunoassays (2).
Case Report: A 46 yo woman with a 5 y h/o fibromyalgia presented with fatigue, headache, anxiety, weight gain (55 pounds over the last 3 y) and generalized pain. PMH included cholecystectomy, partial hysterectomy. FamH was neg for pituitary tumors and included lung tu (mother) and pancreatic ca and renal stones (father). An extensive previous evaluation of the pt had focused on possible Cushing's syndrome. Laboratory did not reveal any abnormalities except an elevated ACTH in January 2008 (261 pg/mL, nl 10-60). The following other hormone measurements were within normal range: prolactin, TSH, free T4, cortisol, estradiol, estriol, pregnenolone, FSH, LH, testosterone and DHEAS. MRI ruled out a pituitary tumor. Repeated high ACTH levels measured initially at UMMC and subsequently at different laboratories (Mayo Clinic, Quest diagnostics) were suspicious. We discussed possible HA interference with our Mayo Clinic and Quest Diagnostics colleagues. Pretreatment of the pt's plasma in a heterophile blocking tube (HBT) resulted in a fall of the ACTH value into the range of normal assays. Non-linearity of ACTH results upon serial dilution further supported HA interference. A different sample was drawn and sent to Quest Diagnostics. After anti-human antimouse antibodies tx the initial high ACTH normalized. Table 1 shows the pt's ACTH results evaluated in our laboratory and at other locations (Mayo Clinic and Quest Diagnostics).
Conclusion: HA are important to consider in the evaluation of pts with obesity and possible CS. Our pt's plasma cross-reacted in ACTH assays at 2 different locations (Mayo Clinic and Quest Diagnostics) leading to falsely elevated results. A mismatch between clinical and initial biochemical data should trigger consideration of HA interference, even in the face of a biochemical test with little previously reported HA interference.

1. Boscato, L.M. & Stuart, M.C. (1986) Incidence and specificity of interference in two-site immunoassays. Clinical Chemistry, 32, 491-495.
2. Jill Tate GW. interferences in immunoassays. Clin Biochem Rev 25:105-120,2004.

Nothing to Disclose: EM, KKN, WAS, CAK

Pub # P3-61

Session Information POSTER SESSION: CLINICAL - Endocrine Tumor Case Reports (1:30 PM-3:30 PM)

Title The Clinical Course of a Non-Islet Cell Tumor Causing Hypoglycemia: A Complex Case of Doege-Potter Syndrome

Author String M Vidyarthi, B Hossain, E Marouf, Z Khatami, ND Stojanovic
South London Healthcare Trust, Farnborough Common, Orpington, UK; South London Healthcare Trust, Farnborough Common, Orpington, UK

Body A 72 year-old man presented with a three-week history of early morning confusion. His capillary blood glucose was 25.2 mg/dl on admission. There was no history of diabetes and he was not on any medications that could account for hypoglycaemia. He had no history of similar episodes previously. Past medical history included resection of a large solitary fibrous tumour of the left pleura in 2002 and 2006 after tumour recurrence. Histology showed a spindle cell tumour with microscopic features of malignancy. Systemic examination was unremarkable except for a left thoracotomy scar. Chest X-ray showed an opacification of the left middle and lower zones. A CT scan of the thorax and abdomen revealed an extensive lobulated mass (18x11cm) in the anterior mediastinum, extending into the left hemithorax. Pancreas was normal. Blood results after an overnight fast were: plasma glucose 34.2 mg/dl, insulin 0.4 mIU/l (3-17), C-Peptide 68 pmol/l, IGF-2 97.3 nmol/l, IGF-2/IGF-1 ratio 19.9 (normal ratio <10). Urinary sulphonylurea screen was negative. A subsequent octreotide scan showed the tumour to be somatostatin receptor positive. The patient was discharged on lanreotide with plasma glucose stable between 59.4 and 140.4 mg/dl. He was readmitted several weeks later with two witnessed generalised tonic-clonic seizures and subsequent right-sided myoclonic movements. He was stabilised acutely on phenytoin. An MRI scan of the brain was normal with an equivocal EEG. Lumbar puncture was normal. Nasogastric feeding was commenced as patient's oral intake was limited exacerbating his hypoglycaemic episodes. Dexamethasone was added to improve his appetite. Phenytoin was stopped as his seizures were attributed to low blood glucose levels, and he had no further episodes. Interferon-alpha was added to treatment with lanreotide. The patient's glycaemic profile has improved, and he was discharged home on interferon-alpha, lanreotide and dexamethasone. The patient was supplied with a continuous glucose monitoring system. He is currently awaiting a surgical opinion. The anti-tumour activity of the interferon family is not wholly understood, but is partially attributed to transcriptional modulation of the IGF system. To the best of our knowledge, this is the first case whereby lanreotide and interferon-alpha has been used to control hypoglycaemia in a patient with IGF2 producing tumour.

Disclosures: NDS: Travel Grant received, Ipsen. Nothing to Disclose: MV, BH, EM, ZK

Pub # P3-62
Session Information POSTER SESSION: CLINICAL - Endocrine Tumor Case Reports (1:30 PM-3:30 PM)
Title Ectopic ACTH Syndrome (EAS), Discrepancy of ACTH Levels, and a Literature Review
Author String F Myint, T Araki, B Alfonso, F Dekio, A Saliby
Beth Israel Medical Center, New York, NY; Beth Israel Medical Center, New York, NY; Beth Israel Medical Center, New York, NY

Body **Introduction:** We report a case of EAS presenting with diabetes insipidus (DI) and a discrepancy in ACTH assays, with a literature review.

Case presentation:

A 75-year-old woman presented with altered mental status and muscle weakness over 2 months. Physical exam revealed decreased proximal muscle strength and pigmented forehead. Labs were remarkable for hypernatremia (157mEq/L) and hypokalemia (3mEq/L). Imaging revealed a right lung mass and hypothalamus metastasis. 3 days later she developed severe polyuria, polydipsia, high serum osmolality (316 mOsm/kg) and urine specific gravity of 1.003, and DDAVP was started. Lung biopsy showed a neuroendocrine tumor, with negative ACTH staining.

Plasma cortisol (50ug/dL) and 24 hr urine cortisol (1560 ug/day) were elevated. ACTH levels were repeatedly in the normal range (20-30 pg/ml) by chemiluminescence assay, but, ACTH was elevated at 115 pg/mL (6-58 pg/ml) by radioimmunoassay (RIA). She failed the 2 day-high dose dexamethasone suppression test. A diagnosis of EAS was made and she was managed with ketoconazole and metyrapone. Cortisol levels decreased after several days. She was transferred to hospice and expired on day 34 after admission.

Discussion:

EAS causes 10% of Cushing's syndrome, half of the cases occurring with small-cell lung cancer (1) (2). It can present with variable atypical features (3). Given the unusual initial presentation with DI, the diagnosis of EAS was challenging.

It is thought that ectopic tumors produce preferentially ACTH precursors (4), and that may result in discrepancies in the levels with different assays. The hypothesis is the difference in assay recognition sites of the various ACTH precursors (5). The bioactivity of ACTH precursors is not well established (6). In our case, the ACTH-cortisol axis was hyperactive given the elevated cortisol levels, and the discrepancy could be due to ACTH precursors or the inappropriate handling/processing of the samples.

Metastasis of tumors to the hypothalamus - pituitary axis is rare, but tends to occur in the hypothalamus or posterior lobe leading to DI (7, 8).

Conclusion:

The diagnosis of EAS can be challenging particularly when associated with other hormonal abnormalities like DI. In the case of pituitary metastasis, DI is the main endocrine manifestation. Discrepancy of ACTH assays can occur and may be explained by overproduction of ACTH precursors.

(1) Wajchenberg B, Journal of Steroid Biochemistry and Molecular Biology 1995; 53: 139-151.

(2) Delisle A, Arch Internal Medicine 1993;153(6): 746-752

(3) Ioannis I, The Journal of Clinical Endocrinology and Metabolism 2005; 90: 4955-4962

(4) Oliver R, Pituitary 2003; 6: 119-126

(5) Tsuchiya K, Endocrine Journal 2005; 52, 743-750

(6) Gewirtz G. Big ACTH, The Journal of Clinical Endocrinology and Metabolism 1973; 38: 227-230

(7) Komninos J, The Journal of Clinical Endocrinology and Metabolism 2004; 89: 574-580

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Nothing to Disclose: FM, TA, BA, FD, AS

Pub # P3-63

Session Information POSTER SESSION: CLINICAL - Endocrine Tumor Case Reports (1:30 PM-3:30 PM)

Title Previously Undescribed LMNA Mutation Associated with Unusual Evolution of a Medullary Thyroid Carcinoma Related to RET 634 Mutation in a 10-Year-Old Patient: A Metabolic or Nuclear Link?

Author String M-C Vantyghem, C Do Cao, A-S Balavoine, P Pigny, J-L Wemeau, O Lascols
Lille University Hospital, Lille, France; Lille University Hospital, Lille, France; H[ocirc]pital Saint-Antoine, Paris, France

Body Background : In children from HMTC families, disease-free survival is best predicted by TNM staging and preoperative basal calcitonin (CT) level <30 pg/ml (1).
Clinical case A 10-year old boy was diagnosed with HMTC because his father had been operated from HMTC, related to RET634 gene mutation in both patients. The boy's mother had a MS. Before surgery, his BMI was 28, with android fat repartition and acanthosis nigricans. The neck palpation was normal. US examination showed a 6-mm hypoechogenic right thyroid nodule. Blood glucose level was 4.78 mmol/l, triglyceride:3.87g/l (N<1.5), total cholesterol: 2.13 (N<2), and HDL :0.29 (N>0.4) g/l. Basal CT level was 33 pg/ml (N<10), peaking to 1790 after pentagatrin. Urine catecholamines were and have remained normal as well as blood and calcium urine. Abdominal CT scan showed liver steatosis. A total thyroidectomy was performed at 11 years, with central and bilateral neck dissection and thymectomy. Histological examination confirmed a bilateral HMTC (right: 7; left: 2mm) with metastases in 4 central and 2 right lymph nodes, 5 of them with capsular rupture. Post-operatively basal CT level remained <3 until 2 years, but then increased progressively: 5 years 38; 7 years 103; 10 years 227 pg/ml. ProCT was 1.3 (N <2). At 13 years, an OGTT was normal (FBG: 4.23; 2-hours-BG: 7.2 mmol/l) despite high insulin (80 mUI/l; N<20), leptin (47 ng/ml;N<12) and ASAT liver enzyme (46UI/l;N<50). At 15 years, the BMI was 34. At 21 years, the BMI decreased to 28 but the cervico-facial fat distribution with calf pseudo-hypertrophy, short legs, small hands (2), and a blood pressure of 130/90 mmHg led to disclose a R156 (H,R) LMNA mutation despite the lack of lipotrophy. Leptin level (7.9 ng/ml) was inappropriate to BMI.
Conclusion: This case reports on a HMTC related to RET634 mutation associated to a MS finally related to a new LMNA mutation responsible for an atypical phenotype (3). Elevated proCT levels, even in the normal range, are associated with MS in the general population, and could have worsened the expression of this laminopathy (4). Otherwise, in the French HMTC series (1), our patient had the lowest CT level among those not cured, all others showing CT above 400. This questions on the role of the laminopathy in the worsening of the HMTC prognosis (growth effect of insulin on C-cells? frailty of their nuclear envelope related to LMNA mutation ?) (5-7), and highlights the difficulty to propose a CT threshold for surgery.

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- 2) Vantyghem Mc et al., J Clin Endocrinol Metab, 2004, 89: 5337-46
- 3) Decaudain A et al., J Clin Endocrinol Metab 2007 92:4835-44
- 4) Abbasi A et al., J Clin Endocrinol Metab. 2010;95:E26-31.
- 5) Vantyghem MC et al., Clin Endocrinol (Oxf).2007;67:247-9.
- 6) Favreau C et al., Exp Cell Res. 2003;282:14-23.
- 7) Fischer AH, et al., Am J Pathol. 2003 ;163:1091-100.

Nothing to Disclose: M-CV, CDC, A-SB, PP, J-LW, OL

Pub # P3-64

Session Information POSTER SESSION: CLINICAL - Endocrine Tumor Case Reports (1:30 PM-3:30 PM)

Title Early Presentation of Bilateral Gonadoblastoma in Denys-Drash Syndrome: A Cautionary Tale for Prophylactic Gonadectomy

Author String PR Patel, J Pappas, BH Franklin, N Arva, PC Brar
New York University School of Medicine, New York, NY; New York University School of Medicine, New York, NY; New York University School of Medicine, New York, NY

Body **Background:** Contiguous gene deletion of the Wilms tumor gene (*WT1*) is associated with two well described syndromes; Denys-Drash (DDS) and Frasier (FS). Both are associated with nephropathy and ambiguous genitalia and have overlapping clinical and molecular features (1). The known risk of Wilms tumors in DDS and gonadoblastomas in FS patients requires tumor surveillance.

Case report: We evaluated a newborn with ambiguous genitalia, intact Mullerian structures (uterus) and small bilateral perivesicular gonads with a 46,XY (SRY+) karyotype. The physical exam revealed labia with clitoromegaly (1.2 cm in length). There were separate urethral and vaginal openings with no urogenital sinus. Labs in the early neonatal period (Quest Diagnostics: all male references) revealed 17-OH-Progesterone 96 (<420 ng/dL), testosterone 133 (2-23 ng/dL; tanner 1), DHEAS 441 (73-367 ug/dL), inhibin A <1 (<21 pg/mL; prepubertal), inhibin B 35 (<161 pg/mL; 3-9 years old), and AMH 3.7 (87.3-243 ng/mL; 1-6 years old). After a joint discussion with the family, geneticist, endocrinologist, and psychologist the infant was assigned a female gender. Based on the phenotype (ambiguous genitalia, pre and postnatal hydronephrosis, and genitourinary abnormalities), the exon and exon-intron boundaries were sequenced. A missense mutation leading to the substitution of lysine for glutamine at position 369 of the WT1 protein (Q369K) in exon 8 was found to be consistent with Denys-Drash syndrome. At seven months of age the patient underwent a clitoroplasty and gonadectomy. Bilateral gonadoblastomas were found in an indeterminate left gonad and a right testis. In addition, the patient had bilateral grade 2-3 vesicoureteral reflux and progressed to end stage renal failure at 11 months of age (creatinine 1.4mg/dl). Consequently, the patient has secondary hyperparathyroidism with PTH 691 (12-65 pg/mL), calcium 7.2 (8-10.4 mg/dL), and phosphorus 7 (2.7-4.5 mg/dL). The patient's most recent renal ultrasound does not show evidence of a Wilms tumor (2).

Conclusion: This is one of the earliest cases of bilateral gonadoblastoma reported in DDS. This case highlights the importance of early gonadectomy at the time of diagnosis of the *WT1* gene mutation as these tumors have potential for malignant transformation.

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Nothing to Disclose: PRP, JP, BHF, NA, PCB

Pub # P3-65
Session Information POSTER SESSION: CLINICAL - Endocrine Tumor Case Reports (1:30 PM-3:30 PM)
Title Immunohistochemical Demonstration of Thyroglobulin in Non-Thyroidal Cancer
Author String V Leyco-Policarpio, B Arafah
University Hospitals Case Medical Center, Cleveland, OH; Case Western Reserve University, Cleveland, OH

Body Thyroglobulin (Tg) is a specific marker of benign and malignant thyroid tissue such that its presence on immunohistological staining of biopsy specimens is used by pathologists to confirm the thyroidal origin of neoplasms. In this report, we present a unique case of metastatic pulmonary carcinoid that stained strongly for Tg.

A 74 year old man with a stable lung nodule for many years presented with 70 pound weight loss over a year and was found to have progression of the lung nodule. PET/CT and bone scans revealed diffuse metastatic disease. Biopsy of the lung mass showed features consistent with adenocarcinoma. On immunohistochemical evaluation, the biopsy specimen was positive for Tg, cytokeratin 7 (CK 7), thyroid transcription factor-1 (TTF-1), and pankeratin but negative for cytokeratin 20 (CK 20) and prostate specific antigen. Appropriate positive and negative controls were utilized in all immunohistochemical staining assays. The patient was referred to us for management of presumed metastatic thyroid cancer. On exam, he appeared euthyroid and had a normal thyroid exam without nodularities. CT scan of the neck was NL. Laboratory data revealed normal T4 and TSH levels. Serum Tg level was 9.7 ng/mL (normal 1.7-56.0) with negative anti-Tg antibodies. The latter measurement was repeatedly confirmed in diluted samples. CEA and chromogranin A serum levels were elevated at 16.7 ug/dL (normal 0-5) and 92.2 ng/mL (normal \leq 36.4), respectively. The immunohistochemical pattern of the patient's biopsy that is positive for CK 7 and TTF-1 and negative for CK 20 is highly suggestive of pulmonary carcinoid tumor. The NL Tg serum level, in the absence of anti-Tg Ab is not consistent with metastatic thyroid Ca. The patient was referred back to the oncology service and was treated with radiation and chemotherapy and died 6 months later.

Immunohistochemical studies are used to distinguish primary sites of carcinoid tumors. TTF-1 staining can be found in 80% of metastatic pulmonary carcinoids and is not seen in the extrapulmonary types (1,2). CK 7 and 20 are likewise expressed in neuroendocrine neoplasms with a report of high specificity of CK 7 for pulmonary carcinoids and CK 20 for those of GI origin(3). Immunohistochemical demonstration of Tg in tumors has historically been synonymous with thyroid cancer. The unique case presented herein indicates exception to this rule and raises concern for potential misclassification of neuroendocrine tumors as thyroid cancer.

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(2) Oliveira Am, Tazelaar HD, Myers JL, Erickson LA, Lloyd RV. Thyroid transcription factor-1 distinguishes metastatic pulmonary from well-differentiated neuroendocrine tumors of other sites. *Am J Surg Pathol.* 2001; 25: 815-819.

(3) Cai YC, Banner B, Glickman J, et al. Cytokeratin 7 and 20 and thyroid transcription factor 1 can help distinguish pulmonary from gastrointestinal carcinoid and pancreatic endocrine tumors. *Hum Pathol.* 2001; 32: 1087-1093.

Nothing to Disclose: VL-P, BA

Pub # P3-66

Session Information POSTER SESSION: CLINICAL - Endocrine Tumor Case Reports (1:30 PM-3:30 PM)

Title Ectopic ACTH-Mediated Cushing Syndrome (CS) from Sporadic Medullary Thyroid Cancer (MTC): An Immunohistochemical (IHC) Confirmation Study

Author String VG Budharaju, JR Howe, P Kirby, RA Robinson, K Kovacs, T O'Dorisio
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Body
Background: Ectopic ACTH mediated Cushing's syndrome (CS) from sporadic Medullary thyroid cancer (MTC) is commonly reported but often not confirmed with Immunohistochemistry (IHC).
Clinical Case: A 62-yr man presented with typical features of CS (upper arm bruising, stria, proximal myopathy and hypertension), and FNA established medullary thyroid cancer (MTC) metastatic to right level III neck lymph nodes. He also had CT evidence of a right adrenal mass with imaging features suggestive of an adenoma. Biochemical studies were consistent with ACTH dependent CS: Serum Cortisol level of 19.9 mcg/dl (6.2 - 19.4 mcg/dl) and ACTH of 81 pg/mL (7-69 pg/mL). 24-hour urine free cortisol was 610 and 54 mcg/24hr (n< 60 mcg/24hr). Serum potassium was 2.8 meq/L (3.5-5.0). CEA was 12.6 ng/mL (0-3). Calcitonin was 131 pg/mL (0-7.5). He underwent total thyroidectomy, with bilateral central neck dissection, and right functional neck dissection (level II-V lymph nodes). Histopathology showed unilateral right thyroid lobe medullary thyroid carcinoma with vascular invasion. All 32 resected positive nodes stained positive for calcitonin, many with extranodal extension. Left thyroid lobe showed multifocal C-cell hyperplasia and ACTH immunostaining was initially reported as negative. Post-operatively, he developed secondary adrenal insufficiency with repeat 24 hr urine free cortisol of 4 mcg/24 hr, morning cortisol was 5.1 mcg/dL and ACTH was 21 pg/mL. Calcitonin fell to 46 pg/mL. As the clinical course was highly suggestive of Ectopic ACTH-mediated CS from the MTC, the paraffin blocks were restained for ACTH and CRH and there was weak but conclusive IHC positivity for ACTH in tumor cells. In a few areas, tumor cells were IHC positive for CRF in the cytoplasm, but were considered negative due to background interference. The CS features dramatically improved after surgery. Unfortunately the patient developed mediastinal lymphadenopathy with rising calcitonin levels. Mediastinal nodal resection confirmed metastatic MTC.
Conclusion: Although some 50 cases of ectopic CS in MTC have been reported in the literature, confirmation with IHC of ACTH or CRH has, to our knowledge, been reported in only one other case. Our case highlights the difficulties faced by clinicians attempting to confirm the source of ectopic ACTH or CRH production, and shows the value that specific IHC can have, as well as the importance of pursuing a clinically suspected diagnosis despite initial negative results.

Nothing to Disclose: VGB, JRH, PK, RAR, KK, TO

Pub # P3-67

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Cancer & Techniques To Study Gene Regulation (1:30 PM-3:30 PM)

Title High Mobility Group Protein 1 (HMGB1) Remodels Nucleosomes and Facilitates Estrogen Receptor Binding

Author String SR Joshi, YA Sarpong, RC Peterson, WM Scovell
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Body Eukaryotic cells have evolved multiple strategies to overcome the repressive nature of chromatin and facilitate essential transactions on DNA. High mobility group protein 1 (HMGB1), a highly conserved and abundant protein in eukaryotic cells, interacts with DNA, nucleosomes and chromatin to influence their structures. These structural changes are associated with the regulatory influence of HMGB1 in transcription, DNA repair and recombination. We show that HMGB1 remodels the canonical nucleosome structure in an ATP-independent manner. The HMGB1-remodeled nucleosomes (N' & N'') remain stable after the removal of HMGB1, exhibit characteristics that are distinctly different from the canonical nucleosome (N) and are similar to those produced by ATP-dependent chromatin remodeling complexes (CRCs). In addition, although estrogen receptor (ER) does not bind to the rotationally phased and translationally positioned consensus estrogen response element (cERE) in the canonical nucleosome, the HMGB1-remodeling of the nucleosome facilitates strong ER binding to its cERE. The collective data support the contention that the canonical nucleosome (N) is a more [ldquo]tense[rdquo], less accessible form (of the nucleosome) than the [ldquo]relaxed[rdquo], more accessible forms (N' & N'') that results from interaction with HMGB1. These findings are considered in terms of the conformational selection model in which the canonical nucleosome can be considered as just one member of an equilibrium ensemble of interconvertible nucleosome states, of which the conformation and functional activity is dependent on the context or the microenvironment of the nucleosome.

Nothing to Disclose: SRJ, YAS, RCP, WMS

Pub # P3-68

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Cancer & Techniques To Study Gene Regulation (1:30 PM-3:30 PM)

Title Differential Expression of MiRNAs in Antiestrogen-Sensitive MCF-7 vs. Antiestrogen-Resistant LY2 Breast Cancer Cells

Author String TT Manavalan, Y Teng, SN Appana, S Datta, TS Kalbfleisch, Y Li, CM Klinge
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Body Aberrant microRNA (miRNA) expression has been reported in breast cancer. The goal of this study was to identify miRNAs differentially expressed in estrogen receptor alpha (ER α)-positive tamoxifen-sensitive MCF-7 *versus* -resistant LY2 human breast cancer cells. Microarray analyses identified 97 miRNAs that are differentially expressed in MCF-7 and LY2 cells under basal conditions. miRNAs expressed in opposite direction between MCF-7 and LY2 cells include miR-10a, -21, -22, -29a, -93, -125b, -181a, -200a, -200b, -200c, -205, -221 and -222. Select results were confirmed using quantitative real time-PCR (qPCR). miR-125b, miR-200a, miR-221 and miR-222 were down-regulated by a 6 h treatment with 100 nM 4-hydroxytamoxifen (4-OHT) in MCF-7 cells and upregulated by 4-OHT in LY2 cells. These results agree with the microarray data. The ER antagonist ICI 162,780 generally blocked the effect of estradiol (E₂) and 4-OHT on regulated miRNAs, *e.g.*, miR-21, miR-181a, and miR-222, indicating that these responses in MCF-7 cells are ER-mediated. Time-dependent variation in basal, E₂, and 4-OHT regulation of selected miRNAs was detected. Bioinformatic analyses to impute the biological significance of the identified miRNAs by identifying their computationally predicted target genes in the human genome using TargetScan, PicTar, and the Sanger miRBase Targets databases was performed and overlapped with established 4-OHT regulated genes in MCF-7 cells. 36 putative mRNA targets were identified. *PDCD4* and *CYP11B1* were selected as mRNA targets for miR-21 and miR-200b/c respectively for further analysis. Since basal miR-21 is higher in MCF-7 than LY2 cells, we expected lower levels of Pdc4 protein in MCF-7 compared to LY2 cells. However, Pdc4 protein was undetectable in LY2 cells. *CYP11B1* mRNA was higher in MCF-7 when compared to LY2 but was not altered by E₂ or 4-OHT. ZEB1, an established target of miR-200 family of miRNAs is expressed in antiestrogen-resistant LY2 cells, but not in MCF-7, data that agree with miR-200 expression in MCF-7 and not in LY2. Further studies will determine whether this differential expression of ZEB1 has a role in tamoxifen-resistance in LY2 cells.

Nothing to Disclose: TTM, YT, SNA, SD, TSK, YL, CMK

Pub # P3-69

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Cancer & Techniques To Study Gene Regulation (1:30 PM-3:30 PM)

Title Integrated Genomic Profiling Identifies Loss of Chromosome 11p Impacting Transcriptomic Activity in Aggressive Pituitary PRL Tumours

Author String M Roche, A Wierinckx, G Raverot, C Legras-Lachuer, S Croze, N Nazaret, C Rey, C Auger, E Jouanneau, P Chanson, J Trouillas, J Lachuer
INSERM U1052; CNRS UMR5286; Lyon Cancerology Research Center, Lyon, France; INSERM U1028; CNRS UMR5292; Lyon Neuroscience Research Center, Lyon, France; Université Lyon 1, Bron, France; U693, Le Kremlin Bic[ecirc]tre, France

Body

CONTEXT
Pituitary adenomas are common intracranial tumors (10-15% of the brain tumors). They are generally considered as benign. However, many (45 to 55%) invade the sphenoid or cavernous sinus and some are described as aggressive with a high proliferation rate and short post-operative time to recurrence. The diagnosis of malignancy is still based on the presence of metastases, therefore the molecular events responsible for tumor progression toward malignant type remain unknown. Integrative multidimensional genomic approach associating DNA structure and transcriptomic analysis can increase the detection and understanding capability of mechanisms related to the aggressiveness of tumors. In human prolactin pituitary tumors different alterations of chromosome structure were identified. However, they have never been correlated with aggressive and malignant types.

METHODS
In order to deciphered whether alterations of chromosome structure is involved in the aggressive and malignant types, we have performed a comparative study combining whole genomic hybridization (Genome wide SNP 6.0 array, Affymetrix) and transcriptomic analysis (Codeword Uniset Human Whole Genome bioarray, Applied Biosystem) of 13 human prolactin pituitary tumors classified as non-aggressive (n=7) and aggressive (n=6) based on clinical, histological and molecular data.

RESULTS
An allelic loss within the p arm region of chromosome 11 was detected in five out of the six aggressive tumors. Moreover, an additional allelic loss in the 11q arm was observed in three of them which were considered as malignant based on the occurrence of metastases during follow-up. Comparison of genomic and transcriptomic data from the same samples showed that the allelic loss on chromosome 11p arm impacted upon the expression of genes located in this imbalanced region. A data filtering strategy allowed us to highlight five deregulated genes (DGKZ, CD44, TSG101, GTF2H1 and HTATIP2) within the missing 11p region potentially responsible for triggering the aggressive and malignant types of prolactin pituitary tumors.

CONCLUSION
This integrative multidimensional analysis combining genomic and transcriptomic data underlines the importance of chromosome allelic loss in determining the aggressiveness and malignancy of tumors. Moreover, this study revealed the potential of integrative multidimensional strategy in the identification of cascade events responsible for the aggressiveness of prolactin pituitary tumors.

Sources of Research Support: Programme Hospitalier de Recherche Clinique National 2006-2008 (no 27-43), the Ligue Contre le Cancer and the Region Rhone-Alpes for financial support.

Disclosures: PC: Advisory Group Member, Eli Lilly & Company; Clinical Researcher, Ipsen; Novartis Pharmaceuticals; Novo Nordisk; Pfizer, Inc.; Merck & Co. Nothing to Disclose: MR, AW, GR, CL-L, SC, NN, CR, CA, EJ, JT, JL

Pub # P3-70

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Cancer & Techniques To Study Gene Regulation (1:30 PM-3:30 PM)

Title Identification of Epigenetic Changes in Postmenopausal Breast Cancer That Contribute to Elevated Intratumoral Estrogen Production

Author String KC Knower, S To, H Pun, Y Miki, K Takagi, H Sasano, E Simpson, C Clyne
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Body The epithelial-stromal microenvironment of breast cancer (BC) results in dramatic gene expression changes compared to normal tissue. Despite these transcriptome changes, genetic alterations are restricted to epithelial cells, underlying the important epigenetic influence on gene regulation in the stroma. Cytochrome aromatase p450, encoded by the gene CYP19A1, catalyzes the synthesis of estrogens from androgens. In postmenopausal women, breast adipose fibroblasts (BAFs) become the major site for estrogen production, in which the distal promoter I.4 (PI.4) drives basal CYP19A1 transcription. In the presence of tumor-derived factors, however, CYP19A1 expression is elevated and is driven by the proximal promoters I.3 (PI.3) and II (PII). The increase in aromatase levels in these cancer-associated fibroblasts (CAFs) contributes to the progression of ER+ tumors. Our previous work has shown that inhibition of DNA methylation increases PI.4-derived CYP19A1 mRNA in BAFs, a similar result also observed in the breast epithelial cell lines MCF10A, MCF-7 and MDA-MB-231, albeit via PII (1). Here we demonstrate that paracrine signaling factors secreted by malignant epithelial cells, such as MCF-7, that up-regulate PII-derived CYP19A1 mRNA in BAFs are epigenetically silenced by DNA methylation in the non-malignant MCF10A cell line. To further our in vitro findings, laser capture microdissection was performed on breast tumor and adjacent normal tissue obtained from women with ER- and ER+ invasive tumors. DNA and RNA isolated from epithelial and CAF cell types were used for sodium bisulfite sequencing and qRT-PCR respectively to identify genomic regions of CYP19A1 and other up-stream regulators, including paracrine signaling factors, that were differentially methylated and expressed between tumors and matched normal tissue. This study uncovers a new layer of complexity in the regulation of aromatase and evokes the possibility that changes in the DNA methylome through tumor progression may give rise to a subsequent increase in aromatase levels in the breast. The use of epigenetic therapies as a selective antagonist for aromatase transcription may therefore provide future avenues in patient care.

(1) Knower KC et al., Mol Cellular Endo 2010; 321(2):123-30

Sources of Research Support: U.S. Department of Defense Post-doctoral Training Award (W81XWH-; 08-BCRP-POSTDOC); Victorian Breast Cancer Research Consortium.

Nothing to Disclose: KCK, ST, HP, YM, KT, HS, ES, CC

Pub # P3-71

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Cancer & Techniques To Study Gene Regulation (1:30 PM-3:30 PM)

Title TLS-CHOP, an Oncogenic Fusion Product in Myxoid Liposarcoma, Attenuates Cell Adhesion Ability through Down-Regulation of Integrin $\alpha 5\beta 1$

Author String S Arai, K Du, R Kurokawa
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Body Myxoid liposarcoma is a neoplasm from mesenchymal origin. Chromosomal translocation of t (12;16) (q13;p11) accompanying TLS-CHOP fusion gene expression is detected in over 90% cases of myxoid liposarcoma patients. Therefore, TLS-CHOP is a useful diagnostic marker for myxoid liposarcoma. Oncogenic property of TLS-CHOP has been indicated by transformation assay using mouse NIH-3T3 cells, but the precise molecular mechanisms of TLS-CHOP for myxoid liposarcoma development still remain to be elucidated. TLS-CHOP is a chimeric protein of an RNA binding protein TLS (translocated in liposarcoma) and a transcription factor CHOP (C/EBP homologous protein) and serves as an abnormal transcription regulator. In this study, we demonstrate the molecular functions of TLS-CHOP regarding gene regulation and cellular growth. Our group revealed recently that two tumor-related genes, cyclin D1 (CCND1) and survivin, were regulated by TLS at transcription level. CCND1-promoter activity was decreased by TLS, whereas survivin promoter activity was enhanced by TLS (1, 2). In luciferase reporter assay, TLS-CHOP exerted different effects, in contrast to TLS, on these promoters. TLS-CHOP enhanced the promoter activity of CCND1. TLS-CHOP could not exert significant effect on survivin promoter activity. These results suggest that TLS-CHOP deregulates gene regulatory systems mediated by TLS, and induces oncogenesis. Next we constructed the stable transformant of TLS-CHOP as a model of myxoid liposarcoma-like cell. It had been reported that TLS-CHOP had cytotoxic effect towards several human cell lines. We obtained similar results using several human cancer cell lines such as HeLa, A549 and MCF7, except for the human embryo kidney-derived cell line 293T. The 293T cells, which stably produced TLS-CHOP (designated as a TLS-CHOP/293T), exhibited slight cellular growth retardation with morphological change. Attenuated cell adhesion activity was observed in TLS-CHOP/293T due to decrease of integrin $\alpha 5\beta 1$. E-cadherin gene, which is other cell adhesion molecule implicated in oncogenesis, was also deregulated by expression of TLS-CHOP. Taken together, our results present a clue to understanding of the roles of TLS-CHOP in the regulation of transcription and cell adhesion of myxoid liposarcoma.

- (1) Wang X et al., Nature 2008; 454:126
(2) Du K et al., Biochem Biophys Res Commun 2011; in press

Nothing to Disclose: SA, KD, RK

Pub # P3-72

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Cancer & Techniques To Study Gene Regulation (1:30 PM-3:30 PM)

Title Bile Acid Is One of the Metabolic Inputs for Hippo Signaling and Results in Spontaneous Liver Tumorigenesis in FXR and SHP Double Mutant Mice

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Body Nuclear receptors Farnesoid X Receptor (FXR) and Small Heterodimer Partner (SHP) are central players in bile acid (BA) metabolism. FXR and SHP (DKO) mice suffer from early onset of liver dysfunction and severe BA accumulation. In addition, the DKO mice phenocopy many key features of Hippo pathway signaling mutants, including increased hepatocyte as well as oval cell proliferation, bigger liver size and spontaneous hepatic carcinogenesis. Hippo - an organ size control pathway culminates with the phosphorylation and inactivation of Yes Associated Protein (YAP). DKO mice exhibit decreased YAP phosphorylation, which strongly correlates with increased liver size, proliferation and spontaneous tumorigenesis. Further, positive YAP targets including Cyclin D1 and Osteopontin are induced in the livers of DKO mice. To investigate how Hippo signaling is compromised in DKO mice we examined the canonical MST1/2 kinases known to phosphorylate YAP, and observed no alteration in them. As MST1/2 double mutants as well as DKO mice showed increased hepatic stem cell proliferation, we examined DDC induced stem cell proliferation model and as expected a decrease in YAP phosphorylation or in other words YAP activation was noted. Interestingly, all three mouse models namely, DKO, DDC diet and MST1/2 double knockout exhibit increase in serum bile acids. Treatment of primary mouse hepatocytes with BAs, clearly indicate that BAs can induce non-canonical YAP activation and act as metabolic input into the Hippo pathway in the liver.

Sources of Research Support: NIDDK.

Nothing to Disclose: SA, MJF, RLJ, DDM

Pub # P3-73

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Cancer & Techniques To Study Gene Regulation (1:30 PM-3:30 PM)

Title Expression of Ghrelin *O*-Acyltransferase (GOAT) mRNA in Prostate Cancer Tissue and Cell Lines and Differential *In Vitro* Regulation of GOAT Expression by Ghrelin and Des-Ghrelin

Author String I Seim, L Amorim, PL Jeffery, AC Herington, LK Chopin
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Body Ghrelin is a 28 amino acid multifunctional peptide hormone that is expressed in the stomach and a range of peripheral tissues, where it frequently acts as an autocrine/paracrine growth factor. Recently, a number of enzymes responsible for the processing of ghrelin have been reported, including the enzyme GOAT (ghrelin *O*-acyltransferase) that adds a fatty acid residue (octanoyl/acyl group) to the third amino acid of ghrelin. The unique acylation of ghrelin is required for it to activate its cognate receptor, the growth hormone secretagogue receptor (GHSR), which mediates many of the endocrine actions of ghrelin. A non-acylated form of ghrelin, des-ghrelin, also circulates in plasma, but does not activate the GHSR and is likely to act via a novel orphan receptor. To produce the mature ghrelin peptide, proghrelin must be processed and this requires the expression of either furin, prohormone convertase (PC)1/3, or PC2. We have previously demonstrated expression of ghrelin and GHSR in prostate cancer and prostate cancer cell lines. Moreover, we have shown that exogenous, acylated ghrelin promotes proliferation in the androgen-independent PC3 prostate cancer cell line and the androgen-dependent LNCaP prostate cancer cell line. In the present study we have demonstrated, using real-time reverse transcription PCR, that GOAT mRNA is expressed at similar levels in normal prostate and human prostate cancer tissue. The RWPE-1 and RWPE-2 normal prostate-derived cell lines and the LNCaP, DU145, 22Rv1 and PC3 prostate cancer cell lines express GOAT and at least one other enzyme necessary to produce mature, acylated ghrelin from proghrelin (PC1/3, PC2 or furin). Finally, we have found that acylated ghrelin, but not des-ghrelin, can directly regulate the expression of GOAT in the PC3 prostate cancer cell line. Ghrelin treatment (100nM) for 6 hours significantly decreased GOAT mRNA expression two fold ($P<0.05$) in the PC3 prostate cancer cell line. Ghrelin did not regulate GOAT expression in the DU145 and LNCaP prostate cancer cell lines, however. This suggests that the regulation of GOAT expression by ghrelin may be cell-type specific. We propose that PC3 may be a useful model cell line to investigate GOAT regulation and function.

Sources of Research Support: Grants from the National Health and Medical Research Council (to LKC, ACH and PLJ), the Cancer Council Queensland (to LKC and ACH), the Faculty of Science and Technology at the Queensland University of Technology (QUT), a QUT International Doctoral Scholarship (to LA), and a QUT Early Career Researcher grant (to IS).

Nothing to Disclose: IS, LA, PLJ, ACH, LKC

Pub # P3-74

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Cancer & Techniques To Study Gene Regulation (1:30 PM-3:30 PM)

Title Stem/Progenitor Cell Gene Expression in Craniopharyngiomas and Pituitary Adenomas

Author String RV Araujo, CMG Cani, MCBV Fragoso, H Matushita, VAS Cescato, MD Bronstein, BB Mendonca, LR Carvalho
IC-Hospital das Clínicas da FMUSP, São Paulo, Brazil; IC-Hospital das Clínicas da FMUSP, São Paulo, Brazil; IPQ-Hospital das Clínicas da FMUSP, São Paulo, Brazil; IC-Hospital das Clínicas da FMUSP, São Paulo, Brazil

Body **Background:** Besides the function of stem cells during pituitary embryonic development and its maintenance during adulthood, stem/progenitor cells connection with pituitary adenomas is an emerging topic. The role of stem/progenitor cells in tumorigenesis is supported by the presence of undifferentiated cellular subpopulation: related with the beginning, development and tumoral maintenance. **Objective:** This work aimed to study the differential expression of stem/progenitor cell genes in craniopharyngiomas and pituitary adenomas. **Methods:** Total RNA of six corticotrophinomas, two somatotrophinomas and two craniopharyngiomas obtained at surgery was used in qRT-PCR analysis of 16 stem/progenitor cell genes (*ABCG2, CD44, DLL4, HESX1, NANOG, NOTCH2, POU5F1, OTX2, MKI67, PITX1, PITX2, POU1F1, PROPI, SOX2, SOX9* and *TBX19*) and adenopituitary hormones genes (*GH, ACTH, PRL, TSHB, FSHB, LHB e CGA*). Human Pituitary Gland - Poly A + RNA (Clontech) was used as control. **Results:** *POU5F1* is 15±4-fold up-regulated in all samples while *DLL4* (a Notch ligand) mRNA levels were increased (11±6-folds) in all pituitary adenomas and in one craniopharyngioma. *HESX1* and *NANOG* presented constitutive expression pattern in pituitary adenomas and down-regulated expression pattern in craniopharyngiomas. *OTX2* expression was undetected in all samples. The mRNA expression of pituitary hormones revealed an increase of ACTH in corticotrophinomas and GH in somatotrophinomas. Pituitary hormones mRNA levels were not detected in craniopharyngiomas. As expected, *POU1F1* mRNA levels were increased in somatotrophinomas, while *TBX19* expression was increased in corticotrophinomas. A surprising finding was *PROPI* up-regulation (22±11-folds) in corticotrophinomas also correlated with increased ACTH mRNA levels in the corresponding samples ($r=0.8$). Further genes analyzed demonstrated heterogeneous expression patterns among samples. **Conclusion:** We found that *POU5F1* and *DLL4* are up-regulated in pituitary tumors and craniopharyngiomas corroborating with previous results described in malignant tumors, such as lung and breast cancer. In addition *PROPI* overexpression in ACTH producing tumors is an intriguing finding and should be explored in order to clarify its real role in corticotrophinomas tumorigenesis.

Sources of Research Support: Fundacao Faculdade de Medicina da Universidade de Sao Paulo.

Nothing to Disclose: RVA, CMGC, MCBVF, HM, VAC, MDB, BBM, LRC

Pub # P3-75

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Cancer & Techniques To Study Gene Regulation (1:30 PM-3:30 PM)

Title Development of *In Vitro* Models To Study the Role of the Tight Junction Protein, Claudin 1, in Breast Cancer Subtypes

Author String AAA Blanchard, LMC Morrison, X Ma, SC Cooper, Y Myal
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Body The claudins are a family of tight junction proteins that are involved in maintaining the differentiated state of epithelial cells. An absence of, or defects in tight junctions have been associated with development of the neoplastic phenotype. The role of claudin 1 in breast cancer is not known or well explored, but recent studies from our laboratory and others suggest that its protein expression is down regulated in human invasive breast cancer and that this down regulation may be associated with an early event in breast cancer progression. Furthermore, we have previously shown that claudin 1 protein expression was significantly higher in the basal-like subtype, a subtype of breast cancers associated with poor prognosis. To begin to delineate a direct functional role for claudin 1 in breast cancer we generated stably transfected shRNA claudin 1 knockdown, and claudin 1 overexpressing *in vitro* models using human breast cancer (HBC) cell lines. Both the T47D and BT20 cell lines endogenously express high levels of claudin 1 and were used for the knockdown models, while the MCF7 and the MDA-MB231 HBC cell lines endogenously express low levels of claudin 1 protein and were used for the overexpressing claudin 1 models. Additionally, the cell lines represent the luminal (T47D, MCF7, favorable prognostic) and the basal (BT20, MDA-MB231, poor prognostic) subtypes of breast cancer. Claudin 1 gene knockdown/overexpression was confirmed by western blot analysis. Cells were then evaluated for proliferation, migration/invasiveness, apoptosis, anchorage independence, and morphological changes.

We have successfully established *in vitro* models to investigate the effects of alterations in claudin 1 gene expression on human breast cancer tumor progression and metastasis. These models will allow us to examine whether claudin 1 has a direct effect on breast tumorigenesis and breast cancer progression. The complex nature of the disease presentation and the limitations in identifying clinically relevant subsets of patients are major challenges for current breast cancer diagnostic and therapeutic strategies. These studies will provide information which would further define existing molecular breast cancer subtypes, and may indicate that claudin 1 has the potential to be an important prognostic biomarker when used in conjunction with existing prognostic indicators.

Nothing to Disclose: AAAB, LMCM, XM, SCC, YM

Pub # P3-76

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Cancer & Techniques To Study Gene Regulation (1:30 PM-3:30 PM)

Title In Silico Promoter Prediction Using Grey Relational Analysis

Author String UD Tatavarthi, NRV Padmanabhuni, AR Allam, SR Gumpeny
GITAM University, Visakhapatnam, India; JNTU, Kakinada, India; Endocrine and Diabetes Centre, Visakhapatnam, India

Body Promoter regions on the chromosome are essential to initiate, modulate and amplify the expression of genes, which are influenced by hormones and other signals. They are identified using in silico methods. Here we employed a new approach to predict the promoter using machine learning algorithm: Grey Relational Analysis (GRA). It was applied to a data set with 2111 samples of promoters and non-promoters of 4 species: Homo Sapiens, Drosophila Melanogaster, Escherichia Coli and Saccharomyces Cerevisiae. The sensitivities and specificities were: 94.9% and 96.54% for Homo Sapiens, 96.4% and 100% for Drosophila Melanogaster, 93.2% and 100% for Escherichia Coli and 98.3% and 94.14% for Saccharomyces Cerevisiae. When compared to those of traditional method, our method demonstrated better prediction accuracy. Thus the proposed GRA is a new method to predict the promoter region. It can be extended to other species promoters.

Nothing to Disclose: UDT, NRVP, ARA, SRG

Pub # P3-77
Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Cancer & Techniques To Study Gene Regulation (1:30 PM-3:30 PM)
Title A Novel Assay To Evaluate the Products of Phage Display
Author String CSF Cheung, JC Lui, J Baron
NICHD, Bethesda, MD

Body Phage display has proven to be a powerful technology for selection of peptides that bind to molecules of interest. A complex mixture of DNA sequences is inserted into a phage genome such that the encoded peptide is displayed on the viral surface. Library members with desired characteristics are enriched by repetitive binding to a target of interest, followed by amplification of the eluted phage. Phage display has important applications in endocrinology, including study of receptor-ligand interactions and identification of novel receptor agonists and antagonists.

After repetitive enrichment, the binding characteristics of selected phage are typically assessed by ELISA. However, this method often lacks sufficient sensitivity and specificity to distinguish phage with increased affinity from controls. We therefore developed a new strategy to assess the ability of selected phage to bind to the target of interest and tested this method using the Ph.D.-7 phage display peptide library. The specific phage clone to be evaluated was mixed in approximately equal amounts with phage lacking insert. This mixture was then incubated with a target sample, allowing the two phage populations to bind. After repeated washing, the remaining bound phage was eluted. Phage DNA was then extracted and PCR performed using a primer pair that amplifies both insertless and insert-containing phage DNA, giving rise to products of two different sizes on agarose gel. We first demonstrated that the band intensity ratio of insertless to insert-containing phage was highly reproducible with a constant ratio over 10,000 fold dilution of input DNA. We then applied this method to assess a specific insert-containing phage that had previously been selected for affinity to epiphyseal cartilage by repetitive enrichment. After mixture with insertless phage, competitive binding, and competitive PCR, a shift in band intensity ratio was observed compared to the input mixture, indicating that the selected phage out-competed the insertless phage. In contrast, analysis of phage not previously selected for target affinity showed a constant band intensity ratio after competitive binding. In conclusion, this novel competitive binding - competitive PCR assay provides a simple and effective method to assess the binding ability of clones selected by phage display.

Sources of Research Support: Intramural Research Program of the Eunice Kennedy Shriver National Institute of Child Health and Human Development, NIH.

Nothing to Disclose: CSFC, JCL, JB

Pub # P3-78

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Inflammation, Stress & Metabolism (1:30 PM-3:30 PM)

Title Transcriptional Regulation during Proinflammatory Responses in Human Cardiomyocytes

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Body Understanding the regulatory processes that control the biology of cardiomyocytes in normal and disease states is required for the development of better preventative and therapeutic approaches to cardiovascular disease (CVD). For example, TNF α signaling is rapidly and chronically propagated in the context of CVD, but the underlying molecular mechanisms are incompletely understood. We are using an established human cardiomyocytes cell line, AC16, to examine proinflammatory molecular responses to TNF α , including the regulation of gene expression. Specifically, we performed Global Run-On and sequencing (GRO-seq) to determine the immediate effects of TNF α signaling on the transcriptome of AC16 cells in response to short treatments (0, 10, 30, and 120 minutes). GRO-seq is a direct sequencing method that provides a [ldquo]map [rdquo] of the position and orientation of all engaged RNA polymerases across the genome at high resolution. We then used a novel bioinformatic approach to identify both coding and non-coding transcripts regulated by TNF α , including mRNAs, rRNAs, tRNAs, microRNAs, and long non-coding RNAs. The key features of the transcriptional profile of the mRNAs are (1) a dramatic down-regulation of the transcription of about 75% of the mRNAs and (2) a rapid and robust up-regulation of a gene set that mediates the proinflammatory response. Gene ontology (GO) analysis indicates that TNF α promotes a transition from a basal growth state to a stress-responsive state.

To investigate the molecular mechanisms that orchestrate this response, we are examining the localization and functions of key players in the pathway, including NF-[kappa]B, CREB1, and poly(ADP-ribose) polymerase-1 (PARP-1) by using global chromatin immunoprecipitation analyses, RNAi-mediated depletion, and chemical inhibition. Our results indicate that human cardiomyocytes dynamically reorganize their transcription factor and gene expression profiles in response to TNF α . NF-[kappa]B is an essential transcriptional regulator during TNF α -mediated inflammatory responses in cardiomyocytes, while CREB1 and PARP-1 facilitate NF-[kappa]B regulatory circuits. Collectively, our studies are helping to elucidate the initiation and propagation of proinflammatory responses in cardiomyocytes. These results should provide new insights into the molecular mechanisms leading to CVD and suggest more effective strategies for treating and preventing CVD.

Sources of Research Support: Grants from the NIH/NIDDK to W.L.K., a predoctoral fellowship from the DOD/BCRP to X.L. and the AHA to R.K., and a postdoctoral fellowship from the PhRMA Foundation to C.G.D.

Nothing to Disclose: XL, CGD, RK, WLK

Pub # P3-79

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Inflammation, Stress & Metabolism (1:30 PM-3:30 PM)

Title Differential Expression of Inflammatory and Metabolic Genes in Adipose Tissue of Patients with Primary Hyperparathyroidism

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Body Primary hyperparathyroidism (PHPT) is characterised by increased production of parathyroid hormone (PTH). The most studied effect of PTH is its influence on calcium metabolism with an elevated concentration of serum calcium and altered bone resorption. In addition to this, patients with PHPT are at increased risk of non-skeletal diseases, such as impaired insulin sensitivity, arterial hypertension and increased risk of death by cardiovascular disease, possibly mediated by a chronic low-grade inflammation. Since patients with PHPT show biochemical signs of a chronic low-grade inflammation, we wanted to investigate whether the adipose tissue reflect this and potentially play an active role. Subcutaneous fat tissue from the neck was sampled from 16 patients with biochemical hyperparathyroidism and from 15 control patients operated for benign thyroid diseases. RNA was extracted and global gene expression was analysed with Illumina BeadArray Technology. We found 608 differentially expressed genes (q -value < 0.05), of which 347 were up-regulated and 261 were down-regulated. Gene ontology analysis showed that PHPT patients expressed increased levels of genes involved in immunity and defence (e.g. matrix metalloproteinase 9, S100 calcium binding protein A9), and reduced levels of genes involved in lipid, fatty acid and steroid metabolism (e.g. fatty acid desaturase 1, stearoyl-CoA desaturase). Our findings demonstrate that PHPT strongly influences gene regulation in fat tissue, which may result in altered adipose tissue function and release of pathogenic factors that increase the risk of cardiovascular disease.

Nothing to Disclose: MHEC, SND, YN, JEV, BA, EAL, GM

Pub # P3-80

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Inflammation, Stress & Metabolism (1:30 PM-3:30 PM)

Title Selective Modulation of the Glucocorticoid Receptor Can Distinguish between Transrepression of NF[κ]B and AP-1

Author String K De Bosscher, I Beck, N Bougarne, D Bracke, I Vanherpe, W Vanden Berghe, G Haegeman
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Body Glucocorticoids (GCs) block inflammation via interference of the Glucocorticoid Receptor (GR) with the pro-inflammatory transcription factors NF- κ B and AP-1, a mechanism known as transrepression. GCs additionally control genes involved in metabolism via a transactivation mechanism. Hence, chronic, supraphysiological GC treatment in immune disorders leads to severe side effects. A controlled restriction of the targeted signaling pathways affected by GR is therefore predicted to result in a higher specificity and selectivity towards immune modulation. Here, we explored how the GR monomer-favoring CpdA affects the activation and activity of AP-1 in fibroblasts. Our results demonstrate that CpdA, unlike classic GCs, selectively blocks NF- κ B- but not AP-1-driven gene expression. CpdA rather sustains AP-1-driven gene expression, a result which is mechanistically explained by the failure of CpdA to block upstream activated JNK kinase and concomitantly also phosphorylation of c-Jun. In concordance, CpdA sustained the expression of the activated AP-1 target gene c-jun, as well as the production of the c-Jun protein. Finally, ChIP analysis clearly demonstrates that only DEX-activated GR, but not CpdA-activated GR, is recruited to AP-1-driven promoters. In conclusion, our data support the hypothesis that a ligand-induced differential conformation of GR may expose different interaction surfaces to yield a different transcription factor cross-talk profile.

Sources of Research Support: We thank the FWO-Vlaanderen for financial support to this research program. In addition, KDB & IB hold a post-doctoral fellowship from the FWO-Vlaanderen.

Nothing to Disclose: KdB, IB, NB, DB, IV, WVB, GH

Pub # P3-81

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Inflammation, Stress & Metabolism (1:30 PM-3:30 PM)

Title Testosterone Imprinting Influences Alcohol-Induced Liver Injury

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Body Sexual dimorphism exists in a variety of liver diseases including hepatocellular carcinoma, hepatitis B virus infection and alcoholic liver disease (ALD). Alcohol metabolism by CYP2E1 can produce toxic byproducts that increase oxidative stress and inflammation. Chronic alcohol exposure can also increase deposition of collagen leading to liver fibrosis/cirrhosis. ALD may progress from early stages of liver steatosis (fat accumulation) to more advanced injury including increased inflammation (steatohepatitis), hepatocyte cell death, fibrosis and ultimately cirrhosis. Prenatal exposure to sex steroid hormones imprints liver genes; however, the effect of testosterone (T) imprinting on CYP2E1 expression and subsequent liver damage has not been investigated. Since a disparity between alcohol susceptibility and progression of liver damage exists between sexes, we hypothesize that sex differences observed in severity of ALD can be attributed to changes in alcohol metabolism as a result of prenatal exposure to T. In the present study, age-matched orchidectomized males (ORCH) and androgenized female rats (AF-produced by T injection on postnatal day 5) were maintained on a Lieber-DeCarli ethanol or isocaloric-matched control diet for 4 weeks. Comparisons were made among four groups: AFs +/- ethanol (no prenatal exposure to T and no ovarian estrogen) and ORCHs +/- ethanol (developmental exposure to T). Exposure to T during development specifically increased the baseline expression of collagen α 1(I) message and CYP2E1 protein which was further increased by ethanol in the adult liver. Additionally, developmental exposure to T increased histological signs of inflammation (inflammatory cell infiltration) and altered the production of several inflammatory serum cytokines including IL-1 α , IL-6 and IL-10 following ethanol exposure. Nuclear factor kappa B (NF[kappa]B) expression was also upregulated in AFs compared to ORCHs suggesting that T imprinting may modulate the NF[kappa]B pathway and expression of target genes which could contribute to the deleterious effects observed in ALD. In conclusion, prenatal exposure to T during liver development may affect baseline gene expression levels, ethanol metabolism and subsequent liver damage predetermining adult susceptibility to alcohol and disease development and progression. Understanding the influence of sex steroid hormone developmental imprinting may provide further insight into sexual dimorphism of ALD.

Sources of Research Support: Carolinas HealthCare Foundation Grant.

Nothing to Disclose: NMS, LWS, WME, AML, JCP, ATH, IHM, HLB, YMH

Pub # P3-82

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Inflammation, Stress & Metabolism (1:30 PM-3:30 PM)

Title Stress Hormones Modulate Thyroid Hormone Action: Synergistic Transactivation of the Krüppel-Like Factor 9 Gene by Thyroid Hormone and Corticosteroid Receptors

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Body Synergistic regulation of target genes by different nuclear receptors (NRs) is an important mechanism for amplifying responses to hormones. A striking example of this occurs during tadpole metamorphosis where glucocorticoid (GC) synergizes with thyroid hormone (TH) to superinduce the expression of TH receptor (TR) target genes, thereby accelerating metamorphosis. This is a mechanism by which the tadpole can accelerate development in response to environmental stress. The transcription factor Krüppel like factor 9 (*Klf9*) is a direct TR and corticosteroid receptor (GR/MR) target gene. *Klf9* is developmentally regulated in frogs and rodents, and one of its functions is as an intermediate of TH action in neuronal morphogenesis. Gene expression analysis in frog and mouse cell lines, and in tadpole and rodent brain showed that TH and GC can synergistically induce *Klf9* expression. The objective of the current study was to investigate the molecular basis for TH and GC synergy on the *Klf9* gene. Transient transfection of a mouse hippocampal cell line (HT22) with 1 kb fragments of the *Xenopus Klf9* flanking region identified a sequence between 6 and 5 kb upstream of the transcription start site that supported synergistic *Klf9* transactivation. Within this region is an evolutionarily conserved fragment of ~180 bp that we named the *Klf9* synergy module. We show that the homologous region of the mouse *Klf9* gene also supports hormone synergy. Computer analysis identified several putative NR half sites in the *Klf9* synergy module to which TR and GR/MR may bind. We show by electrophoretic mobility shift, DNaseI footprinting and chromatin immunoprecipitation (ChIP) assay that TR and GR bind to specific sequences within the mouse *Klf9* synergy module. In addition, we show by ChIP assay that combined treatment of HT22 cells with TH and GC leads to hyperacetylation of histone 4 at the region of the synergy module. These data suggest that hormone-bound TR and GR/MR may cooperate to promote recruitment of histone acetyl transferases (HAT) or may enhance existing HAT activity. We are now investigating whether combined hormone treatment promotes recruitment of NRs and coactivators to the synergy module to understand the molecular basis for hormone synergy. Synergistic transactivation of target genes may be a common mechanism among vertebrates for stress hormones to modulate the developmental actions of TH.

Sources of Research Support: NIH grant 1 R01 NS046690 awarded to RJD; Rackham predoctoral fellowship awarded to PB.

Nothing to Disclose: PB, RJD

Pub # P3-83

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Inflammation, Stress & Metabolism (1:30 PM-3:30 PM)

Title Characterization of the Calbindin-D28k Gene Proximal Promoter Activity and Regulatory Elements under Charcoal Stripped or Serum-Free Stress Conditions

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Body **Introduction:** Calbindin-D28k (D28k) is a cytosolic calcium binding protein mainly expressed in kidney and brain but also in several other tissues. As an antiapoptotic factor, D28k protects the cell by buffering excessive intracellular free calcium. Reports indicate that certain physiological conditions may be involved in regulation of calbindin-D28k expression. For example, animals suffering prolonged subordination stress showed an elevation of intracellular calcium ions and disruption of neuronal calcium homeostasis accompanied by upregulation of D28k gene. The objectives of present study are to determine whether the proximal promoter of the D28k gene is responsive to serum deprivation as an in vitro model of stress conditions and to characterize the promoter activity and regulatory elements important to the response.

Methods: A chimeric gene construct was prepared by using ~3.2kb 5'-flanking region (-3036/+139) as a full promoter of mouse calbindin-D28k gene ligated up-stream of the luciferase reporter gene. To measure the promoter activity under normal and stress condition, this chimeric reporter plasmid was used for transient transfection studies in HEK-293 and MDCK cells grown in normal fetal bovine serum (FBS), charcoal stripped serum (csFBS) or serum-free medium (SFM). For further characterization of the promoter, several deletion constructs were prepared corresponding to -915/+139, -140/+139 and -3036/+1.

Results: Relative to the empty vector, D28k promoter was expressed in both MDCK and HEK-293 cells under FBS, csFBS or SFM conditions. During transient transfection in MDCK cells, the promoter activity increased with decreasing serum concentrations or cells grown in csFBS. Compared to full promoter, the proximal promoter activity, under csFBS conditions, is significantly decreased when HEK-293 cells were transfected with chimeric plasmids corresponding to -915/+139 and -140/+139 promoter regions. Interestingly, in HEK cells grown in csFBS, promoter activity fell to background levels of the empty vector upon deletion of 139bp downstream from start site. Based on these results we conclude: a) Calbindin-D28k expression is upregulated under stress of serum deprivation and b) more than 1 region downstream from start site of the proximal promoter is important for the promoter activity response to csFBS. Further study is underway to identify the cis-acting elements in downstream region (+1/+139).

Nothing to Disclose: AH, OKO

Pub # P3-84

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Inflammation, Stress & Metabolism (1:30 PM-3:30 PM)

Title Regulated Expression of the Plasminogen Activator Inhibitor Type 1 Gene Requires CCAAT/Enhancer-Binding Protein α

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Body Plasminogen activator inhibitor type 1 (PAI-1) is an acute response factor that is rapidly produced by many types of cells in response to local injury or systemic stress. Rapid degradation of the protein indicates that regulation is transcriptional. However, a consensus regarding PAI-1 promoter activation has not emerged despite intense study. Many stress responsive factors such as interleukins and insulin activate the proximal PAI-1 promoter at a composite element combining AP-1 and Forkhead binding sequences that we call the multi response element (-60/-42). Our studies suggest that CCAAT enhancer binding protein α (C/EBP α) is required for stimulation of PAI-1 by agents acting at this multi response element. Overexpression of C/EBP α increased basal and factor-stimulated PAI-1 luciferase expression 10-fold while expression of Chop 10, an inhibitor of C/EBP α , decreased basal expression and almost totally inhibited stimulated-PAI-1-luciferase expression. These results were supported by lentiviral-mediated knockdown of C/EBP α that also blocked PAI-1 mRNA production of the endogenous gene by insulin and cAMP. Analysis using linker-scanning mutants shows that the activity of C/EBP α is in the multi response element and not at a previously suggested upstream element. ChIP assay showed the binding of C/EBP α to this region of the PAI-1 promoter. Pull-down assays show that insulin increases the dimerization of C/EBP α . Insulin also increases the phosphorylation of C/EBP α on S21 and T222/226. Mutagenesis of these sites will indicate if the increased dimerization results from increased phosphorylation. In contrast to LexA-FoxO3a, LexA- C/EBP α constructs are not responsive to insulin or cAMP although they do have significant constitutive activity. This suggests that C/EBP α is a necessary accessory factor in the activation of PAI-1 gene expression by insulin or cAMP and it may be the broadly expressed transcription factor that supports the expression of this gene in so many cell types.

Nothing to Disclose: FMS

Pub # P3-85

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Inflammation, Stress & Metabolism (1:30 PM-3:30 PM)

Title Novel Interacting Proteins for Steroidogenic Factor-1 (SF-1, NR5A1, Ad4BP) in the Developing Human Adrenal Gland

Author String L Lin, R Parnaik, B Ferraz-de-Souza, JC Achermann
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Body Steroidogenic factor-1 (SF-1, NR5A1, Ad4BP) is a nuclear receptor transcription factor that plays a key role in adrenal and reproductive development. Although several potential SF-1 interacting proteins have been reported in the past 15 years, the identification of interacting proteins related to specific tissues or developmental stages is limited. To address this, a yeast two-hybrid screen for SF-1 interacting proteins was performed using a cDNA library constructed from human fetal adrenal tissue at a critical early stage of development (7 weeks post conception) when SF-1 is strongly expressed and transcriptionally active. The bait for the yeast two-hybrid screen was constructed by fusing the putative ligand-binding region of human SF-1 (codons 221-461) to a yeast DNA-binding domain that activates the UAS promoter and allows nutritional and beta-galactosidase selection. Following cross mating of yeast strains, stringent nutritional marker selection and elimination of potential false positive results, a subset of candidate factors was identified (RING1, ZG16B, FLNA, CIR, CUL7, HSCARG). These factors all showed nuclear localization together with GFP-tagged SF-1 in transfected cells. Furthermore, the potential interaction of these factors with SF-1 was validated using Duolink^{reg} in situ Proximity Ligation Assays, which allow qualitative and quantitative analysis of protein-protein interactions in tissues and cells. Further studies of three of these factors (RING1, ZG16B, FLNA) showed an augmentation of SF-1-dependent transcriptional regulation in NCI-H295R adrenal cells using an SF-1-responsive promoter (ANGPT2), and expression of these proteins during human fetal adrenal development was shown by immunohistochemistry. Taken together, these results suggest that additional novel SF-1-interacting proteins exist. However, it remains to be established whether these represent tissue-specific co-regulators rather than more ubiquitous components of general transcriptional machinery.

Sources of Research Support: The Wellcome Trust (079666).

Nothing to Disclose: LL, RP, BF-d-S, JCA

Pub # P3-86

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Inflammation, Stress & Metabolism (1:30 PM-3:30 PM)

Title The Transcription Factor FoxO4 Represses Cholesterol and Fatty Acid Biosyntheses by Acting as a Cofactor to Sterol Regulatory Element Binding Proteins SREBP2 and SREBP1c

Author String J Zhu, F Chehab
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Body Forkhead proteins (FOX) constitute a large family of transcription factors, of which FoxO1, FoxO3a, FoxO4 and FoxO6 represent the O group. FoxOs bind to DAF-16 binding elements (DBE) in the promoters of multiple genes to regulate apoptosis, cell-cycle control, oxidative stress and longevity. We recently reported that FoxO4 represses cholesterol biosynthesis by acting on the lanosterol 14 α demethylase (known as CYP51) promoter at the late steps of cholesterol biosynthesis. Using luciferase reporter gene assays, electromobility shift, co-immunoprecipitation and chromatin immunoprecipitation assays, we unveiled the mechanism by which FoxO4 represses transcriptional regulation. We show that FoxO4 interacts with the sterol regulatory factor SREBP2 to stimulate CYP51, HMG coA synthase and HMG coA reductase expression. However, FoxO4 also physically interacts with the hypoxia inducible factor HIF2 α and together with SREBP2 forms a ternary complex that represses transcriptional activity at these promoters. EMSAs also demonstrate that at the CYP51 promoter, SREBP2 and HIF2 α bind to their respective SRE and HRE DNA binding sites, which are 4 bp apart. However, FoxO4 binds to its DBE element 303 bp upstream of SRE and HRE. Added together and based on the CYP51 promoter, these findings propose two models: First, each transcription factor binds to its DNA binding site until a favorable chromatin configuration recruits FoxO4 to SREBP2 and HIF2 α to form the SREBP2/FoxO4/HIF2 α complex. In the second model, FoxO4 would be recruited directly to the DNA-bound SREBP2 and HIF2 α independent of DBE binding, thus acting as a direct cofactor. We also found that HIF2 α is stable under normoxic conditions and FoxO4 gene expression is induced during hypoxia. Thus, FoxO4, SREBP2 and HIF2 α regulate cholesterol biosynthesis in normoxic and hypoxic conditions, thereby broadening their roles in metabolism. In addition, we determined that FoxO4 represses promoter activity of the fatty acid synthase and acetyl-coA carboxylase genes. However, unlike with SREBP2 and HIF2 α , FoxO4 interacts solely with the sterol regulatory factor SREBP1c to mediate this repression. Altogether, these studies demonstrate that FoxO4 is a key player in regulating cholesterol and fatty acid biosyntheses and as such paves the way to its use or of its analogs as therapeutic agents for the treatment of disorders caused by a dysregulation of cholesterol and fatty acids biosyntheses.

Nothing to Disclose: JZ, FC

Pub # P3-87

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Inflammation, Stress & Metabolism (1:30 PM-3:30 PM)

Title Identification of the Promoter Region for Cytochrome P450 Oxidoreductase Gene

Author String S Soneda, M Fukami, T Ogata
National Research Institute for Child Health and Development, Tokyo, Japan

Body [Background] Cytochrome P450 oxidoreductase (POR) deficiency (PORD) is a rare autosomal recessive disorder characterized by skeletal dysplasia referred to as Antley-Bixler syndrome, adrenal dysfunction, 46,XY and 46,XX disorders of sex development, and maternal virilization during pregnancy. This condition is caused by mutations in the POR gene encoding an electron donor for all microsomal P450 enzymes and several non-P450 enzymes. Although POR is known to consist of at least a single non-coding exon 1 and coding exons 2-16, its promoter region remain to be identified.

[Purpose] To report the putative promoter region of POR.

[Patients] We studied three Japanese patients with PORD (cases 1-3) in whom DNA sequencing analysis showed heterozygosity for the R457H mutation and RT-PCR sequencing analysis revealed transcription failure of the apparently normal alleles.

[Molecular studies] Comparative genomic hybridization analysis was performed using leukocyte genomic DNA, identifying a microdeletion encompassing exon 1 of case 1, and microdeletions involving exons 1 and 2 of cases 2 and 3. Subsequent sequencing of long PCR products obtained with primers flanking the deleted regions revealed a 2,487 bp deletion in case 1 and the same 49,604 bp deletion in cases 2 and 3.

[In silico analysis] Three putative Sp1 binding sites (sites A-C from the transcription start site) were identified within the 2,487 bp deleted sequence common to cases 1-3.

[Methylation analysis] Bisulfite methods demonstrated virtually unmethylated patterns of sites A-C.

[Electrophoretic mobility shift assay (EMSA) and luciferase assay] Sp1 protein was shown to bind to the three putative Sp1 binding sites A-C by EMSA. Furthermore, relative luciferase activity was markedly increased (~120 times) by using a reporter construct with a ~277 bp fragment harboring sites A-C, whereas deletions of site A, site B, site C, and sites A+B+C decreased luciferase activities by ~80%, ~15%, ~5%, and nearly 100% respectively.

[Conclusion] The present study implies that the region encompassing the three unmethylated Sp1 binding site functions as the component of promoter for POR, as has been reported for rat Por. Since Sp1 binding sites are often identified in the promoter regions of housekeeping genes, this would also be consistent with ubiquitous expression of POR. Further studies will permit to understand the promoter apparatus for POR.

Nothing to Disclose: SS, MF, TO

Pub # P3-88

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Inflammation, Stress & Metabolism (1:30 PM-3:30 PM)

Title Nandrolone Increases Expression of PGC-1 α and Genes of Oxidative Metabolism in Muscle Paralyzed by Spinal Cord Injury

Author String CP Cardozo, Y Wu, J Pan, WA Bauman
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Body Nandrolone, an anabolic steroid, reduces muscle atrophy due to immobilization or nerve transection, and testosterone reduces muscle atrophy after spinal cord injury (SCI), a condition which is associated with increased adiposity, insulin resistance, and carbohydrate intolerance. The molecular mechanisms responsible for this effect are not well understood. In other models of muscle atrophy, testosterone and nandrolone reduce expression of genes promoting muscle atrophy. In glucocorticoid-induced muscle atrophy, testosterone reduces expression of atrophy-inducing genes and increases levels and nuclear entry of the transcriptional co-regulator PGC-1 α , a key determinant of oxidative metabolism and muscle fiber phenotype. In this study, the effects of nandrolone were examined on muscle mass and gene expression in a rat model of hindlimb paralysis due to transection of the mid-thoracic spinal cord in which voluntary movement is absent, and reflex and spastic movements are minimal. One group of animals had SCI alone, and the other, SCI and nandrolone administration by continuous infusion which was begun on the day of the spinal cord transection. A third group of animals had a Sham-SCI surgery in which the spinal cord was exposed but not transected. The muscle tissues were analyzed 56 days thereafter. Gastrocnemius weights were reduced by ~50% by SCI, whereas nandrolone administration prevented ~40% of this loss. Nandrolone did not alter expression of genes promoting muscle atrophy (MAFbx, MuRF1, FOXO1 or FOXO3A) or muscle gain (IGF-1, IGF-1R); expression of these genes was not altered by SCI. Expression of mRNA for PGC-1 α and PGC-1 β were markedly reduced by SCI, as was that for the PGC-1 α target genes LDH-A, medium chain acyl-CoA dehydrogenase, succinyl-CoA:3-ketoacid-coenzyme A transferase, slow myosin, and troponin C1-slow. Nandrolone significantly increased the expression of PGC-1 α and medium chain acyl-CoA dehydrogenase mRNA; significant increases in LDH-A protein and troponin I-slow protein were also observed. These findings indicate that nandrolone stimulates the expression of PGC-1 α and suggest that the protein thus expressed is transcriptionally active and capable of increasing levels of at least some of the proteins that are required for oxidative metabolism of skeletal muscle.

Sources of Research Support: Veterans Health Administration, Rehabilitation Research and Development Service (grants B4162C, B4616R and B3347K).

Nothing to Disclose: CPC, YW, JP, WAB

Pub # P3-89

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Inflammation, Stress & Metabolism (1:30 PM-3:30 PM)

Title Estrogen Receptor Beta Inhibits Transcriptional Activity of Hypoxia Inducible Factor-1 through the Downregulation of Arylhydrocarbon Receptor Nuclear Translocator

Author String C Park, J Park, M Shim, Y Lee
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Body Estrogen receptor (ER) β is predicted to play an important role in prevention of breast cancer development and metastasis. We have shown previously that ER β inhibits hypoxia inducible factor (HIF)-1 α mediated transcription, but the mechanism by which ER β works to exert this effect is not understood. Vascular endothelial growth factor (VEGF) was measured in conditioned medium by enzyme-linked immunosorbent assays. RT-PCR, Western blotting, immunoprecipitation, luciferase assays and chromatin immunoprecipitation (ChIP) assays were used to ascertain the implication of ER β on HIF-1 function. In this study, we found that the inhibition of HIF-1 activity by ER β expression was correlated with ER β 's ability to degrade aryl hydrocarbon receptor nuclear translocator (ARNT) via ubiquitination processes leading to the reduction of active HIF-1 α /ARNT complexes. HIF-1 repression by ER β was rescued by overexpression of ARNT as examined by HRE-driven luciferase assays. We show further that ER β attenuated the hypoxic induction of VEGF mRNA by directly decreasing HIF-1 α binding to the VEGF gene promoter. These results show that ER β suppresses HIF-1 α -mediated transcription via ARNT downregulation, which may account for the tumor suppressive function of ER β .

Nothing to Disclose: CP, JP, MS, YJL

Pub # P3-90

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Gene Regulation: Inflammation, Stress & Metabolism (1:30 PM-3:30 PM)

Title Glucocorticoids Regulate Gene Expression by a Novel Derepression Mechanism Involving Dismissal of the Hes1 Repressor

Author String J Revollo, J Cidlowski
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Body Hes1 is a highly conserved basic helix-loop-helix transcription factor that is necessary for mammalian life. While it has been shown to be an essential component of development and organogenesis across species, little is known of its role in adult tissues. Molecularly, Hes1 functions as a transcriptional repressor by binding to DNA elements in gene promoters called N-boxes (CACNAG), and recruiting chromatin-modifying factors to target genes. Glucocorticoids are steroid hormones that allow us to cope with a variety of environmental and physiological stresses via the ubiquitously expressed glucocorticoid receptor (GR). We now demonstrate that glucocorticoid signaling is able to rapidly silence the expression of Hes1 at both mRNA and protein level, and that this silencing is necessary to initiate a large number of glucocorticoid-mediated changes in gene expression. The mechanism of action by which glucocorticoids repress Hes1 involves an interaction of the GR with a newly identified Nuclear Factor Kappa B (NFkB) site at the Hes1 promoter. Overexpression of Hes1 in human cells leads to a profound reduction of glucocorticoid-mediated changes in gene expression, whereas knockdown of Hes1 results in increased sensitivity to glucocorticoids. To study the role of Hes1 in vivo, we have created mice bearing loxP sites in the Hes1 gene for tissue and time specific knockout experiments, and we have deleted Hes1 in the adult liver using Albumin-Cre mice. Absence of Hes1 in adult liver resulted in no gross physiological or morphological defects. However, these liver Hes1 deficient mice exhibited impaired glucose tolerance and abnormal gene expression patterns in the liver. Surgical removal of the adrenal glands demonstrated that these phenotypes were dependent on the presence of glucocorticoids. Our results identify Hes1 as a new component of the GR pathway whose silencing is necessary to elicit glucocorticoid signaling.

Sources of Research Support: NIH.

Nothing to Disclose: JR, JC

Pub # P3-91

Session Information POSTER SESSION: CLINICAL - Parathyroid Hormone & Parathyroid Hormone-Related Peptide II (1:30 PM-3:30 PM)

Title Response to Denosumab in Refractory Hyperparathyroidism of Parathyromatosis/Parathyroid Carcinoma

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Body **Background:** A rare cause of recurrent hyperparathyroidism (HPT) is parathyromatosis, characterized by multiple foci of hyperfunctioning parathyroid tissue in the neck or mediastinum. Histologically benign, its tenacious seeding and recurrence is similar to parathyroid carcinoma, making them difficult to differentiate. Treating refractory hypercalcemia in this setting is challenging.

Clinical case: A 41 y.o. female presented with severe HPT (Ca 16.4mg/dL, n< 10.5; intact PTH 1092pg/mL, n<65). Excision of a 3.5cm left lower benign parathyroid tumor achieved apparent cure. Nine years later, HPT recurred with Ca 11.7, PTH 97. Surgery yielded 3 histologically benign parathyroid fragments, one of which surrounded but did not invade the recurrent laryngeal nerve, consistent with parathyromatosis; PTH fell from 200 to 36. BMD showed osteoporosis (T= -3.2) at the spine. HPT recurred at age 52, cinacalcet was started and increased as tolerated to > 90mg/d. As Ca rose to >12mg/dL, multiple repeated infusions of zoledronic acid or pamidronate were added but achieved little clinical or biochemical response. Experimental PTH fragment immunotherapy was unsuccessful, as was a third neck exploration which only decreased PTH from 1355 to 1192pg/ml. Vascular invasion was seen on frozen section, suggesting parathyroid carcinoma, but was not found on permanent sections.

Upon its approval for osteoporosis, denosumab 60mg sc was administered. Pre-treatment serum Ca was 12.7mg/dL, PTH 1260pg/mL, urine NTX 777 (19-63) and bone specific alkaline phosphatase (BSAP) 258 (<24). Within 5 days, she had robust decreases in Ca (13.3 to 9.0-10.2), urine NTX (777 to 14), BSAP (258 to 192), with marked relief of hypercalcemic symptoms. cinacalcet was stopped. PTH levels rose by 43%.

Suppression was maintained for 3 months; a 2nd dose was given at 3 months when Ca had risen to 13.5, with similar biochemical/clinical response.

Conclusion: This is the first case demonstrating successful (albeit short-term to date) use of denosumab, a RANK-L antibody, in treating persistent PTH-driven hypercalcemia which had been resistant to cinacalcet and bisphosphonate therapy. Denosumab clearly differs in mechanism when compared to bisphosphonates and further studies needed to understand this difference on osteoclast/osteoblast activity. Denosumab may, in the future, also provide a new treatment for resistant hypercalcemia.

Nothing to Disclose: AV, JK, AA

Pub # P3-92

Session Information POSTER SESSION: CLINICAL - Parathyroid Hormone & Parathyroid Hormone-Related Peptide II (1:30 PM-3:30 PM)

Title Development of Tertiary Hyperparathyroidism in Suboptimally Treated Patients with Pseudohypoparathyroidism Type IB

Author String D El-Maouche, NM Neary, SK Libutti, AM Moses, LS Weinstein
NIDDK, NIH, Bethesda, MD; NICHD, NIH, Bethesda, MD; NCI, NIH, Bethesda, MD; SUNY Upstate Medical Center, Syracuse, NY; NIDDK, NIH, Bethesda, MD

Body Background: Pseudohypoparathyroidism type 1B (PHP1B) is characterized by PTH resistance at the proximal renal tubule in the absence of other endocrine or physical abnormalities. As PTH responsiveness is preserved in bone, these patients can develop skeletal effects of secondary hyperparathyroidism if treated suboptimally with calcium/vitamin D. Insufficiently treated patients are theoretically also at risk for developing tertiary hyperparathyroidism.

Case Series: Five female PHP1B (2 sisters and 3 unrelated) patients with no features of Albright hereditary osteodystrophy or thyroid dysfunction initially presented with symptomatic hypocalcemia and hyperphosphatemia between the ages of 12-20 (median 16). In 3 out of 3 patients, urinary cAMP levels showed a minimal response to exogenous PTH analog. In all 5 patients the diagnosis of PHP1B was confirmed by the absence of *GNAS* exon 1A methylation. The patients were treated with calcium and vitamin D but eventually over a period of 21-42 years from diagnosis (median 34) they developed concomitantly elevated serum calcium and PTH levels (range 254-864 pg/ml), consistent with tertiary hyperparathyroidism requiring discontinuation of calcium and vitamin D. All patients had one or two lesions suspicious for enlarged parathyroid gland on neck ultrasound, and at least one area of increased uptake on sestamibi scan. Four patients underwent parathyroidectomy with removal of a single very enlarged gland from 2 patients and removal of 2 enlarged glands from the 2 other patients. Pathology was consistent with parathyroid adenoma for all resected glands. Calcium and/or vitamin D therapy was reinstated postoperatively and at 93 months median follow-up, PTH levels ranged between 92-119 pg/ml (normal < 87 pg/ml).

Conclusion: PHP1B patients may eventually develop tertiary hyperparathyroidism requiring the removal of autonomous parathyroid adenomas to reverse hypercalcemia and allow reinstatement of therapy for underlying PTH resistance. PHP1B patients are also known to develop significant skeletal manifestations resulting from chronically elevated PTH levels. As both of these complications may result from suboptimally treated secondary hyperparathyroidism and these patients generally do not develop hypercalciuria with treatment, we recommend aggressive management of PHP1B patients with calcium/vitamin D to try to normalize PTH levels, if possible, while avoiding hypercalciuria.

Sources of Research Support: Intramural Research Program, NIH.

Nothing to Disclose: DE-M, NMN, SKL, AMM, LSW

Pub # P3-93

Session Information POSTER SESSION: CLINICAL - Parathyroid Hormone & Parathyroid Hormone-Related Peptide II (1:30 PM-3:30 PM)

Title Osteocalcin and Glucose Homeostasis in Hypoparathyroidism

Author String R Ives, J Sliney Jr, J Korner, DJ McMahon, JP Bilezikian, G Kovalevskaya, C Zhang, E Dworakowski, S Cremers, MR Rubin
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Body Undercarboxylated osteocalcin (uOCN), a bone-specific molecule, was recently shown to improve glucose homeostasis in mice, demonstrating a previously unknown link between bone remodeling and energy metabolism. Whether uOCN similarly affects glucose homeostasis in humans is unknown. In Hypoparathyroidism (HypoPT), a disorder of absent parathyroid hormone (PTH), bone remodeling and uOCN levels are low, but increase with PTH administration. It is thus a model in which metabolic parameters can be tracked along with a change in uOCN levels. We hypothesized that with PTH replacement in HypoPT, increases in uOCN would be associated with a concomitant improvement in glucose homeostasis. 8 HypoPT subjects (7 female, 44 ± 16 yr, BMI 28 ± 4 kg/m², serum calcium 9.0 ± 0.8 mg/dl, PTH <3 pg/ml) were treated with PTH(1-84) 100 mcg qod for 1 yr. Measurements of uOCN (Takara Pharmaceuticals, nl premenopausal range 0.67-1.80 nmol/L), fasting glucose, insulin and C-peptide, as well as oral glucose tolerance testing (oGTT) were performed at 0, 1, 3, 6 and 12 months, along with calculation of the HOMA-IR a measure of insulin resistance. With PTH(1-84), uOCN increased from baseline (1.58 ± 0.9 nmol/L) to 3 mo (2.94 ± 1.6 nmol/L, $p=0.05$), to 6 mo (3.09 ± 0.9 nmol/L, $p=0.002$) and to 12 mo (2.13 ± 1.0 nmol/L, $p=0.03$). At 6 mo, the fasting insulin decreased from baseline (8.70 ± 2.4 to 7.04 ± 2.3 mIU/ml, $p=0.04$) as did the HOMA-IR (2.1 ± 0.6 to 1.7 ± 0.5 , $p=0.01$); at 12 months, the fasting glucose decreased from baseline (98.9 ± 8 to 88.5 ± 6 mg/dl, $p=0.003$), as did the HOMA-IR (1.4 ± 0.4 , $p=0.02$) and C-peptide levels (2.68 ± 0.8 to 2.28 ± 0.7 ng/ml, $p=0.05$). With oGTT testing, at 12 mo the 30 min glucose level was lower than the baseline 30 min level (167.2 ± 18 to 120.5 ± 37 mg/dl, $p=0.02$) and the 120 min insulin level was trended to be lower than the baseline 120 min level (46.8 ± 29 to 20.9 ± 16 mIU/ml, $p=0.07$). These data suggest that PTH-regulated increases in uOCN in HypoPT are associated with parameters that suggest enhanced insulin sensitivity.

Sources of Research Support: CTSA/CTO Pilot Grant Columbia University.

Nothing to Disclose: RI, JS, JK, DJM, JPB, GK, CZ, ED, SC, MRR

Pub # P3-94

Session Information POSTER SESSION: CLINICAL - Parathyroid Hormone & Parathyroid Hormone-Related Peptide II (1:30 PM-3:30 PM)

Title Long-Term Follow-up of a Clinical, Laboratory and Molecular Assessment of a Woman with Jansen Chondrodysplasia Due to a *De Novo* Mutation in PTHR1

Author String DF Carvalho, L Onuchic, S Bertoncetto, BB Mendonca, PHS Correa, RM Martin
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Body **Background:** Jansen's metaphyseal chondrodysplasia (JMC) is a rare autosomal dominant disorder characterized by severe skeletal abnormalities, involving growth plates, leading to short limb dwarfism. Even though levels of PTH are low, patients with JMC normally present asymptomatic hypercalcemia and hypophosphatemia. This condition is the result of activating mutations, in heterozygous state, on the PTH receptor (PTHR1). Up to now, missense mutations have been described involving only the codons 223, 410 and 458 of PTHR1. Long term follow up and treatment of these patients are not described. **Case Report:** We followed the patient SOS (currently 26 yo) who, at birth, presented muscular hypotonia and skeletal abnormalities. At the age of 4, she came to HC-FMUSP because of short stature and growth failure. Based on her laboratorial findings (hypercalcemia, normal phosphorus and creatinine levels, elevated urinary cyclic AMP and high calcium/creatinine clearance) and skeletal deformities, the JMC diagnosis was disclosed. Nowadays, her height is 125 cm after orthopedic surgery and she has developed nephrolithiasis probably secondary to hypercalcemia and hypercalciuria. In order to reduce the hypercalciuria, 10 mg/d of alendronate was prescribed without improvement. Surprisingly, despite long-term hypercalcemia, her calcium-score (measured by coronary CT angiography) was zero. Genomic DNA was extracted from blood cells and amplification of exons 7 (codon 223), 12 (codon 410) and 13 (codon 458) of PTHR1 gene by PCR was done. Direct sequencing of the fragments generated by PCR revealed the mutation H223R on PTHR1 in heterozygous state, confirming the JCM diagnosis. It is a [ldquo]de novo[rdquo] mutation, since the same mutation was not found in the genomic DNA of her parents. **Conclusion:** To our knowledge, this is the longest and well characterized followed-up of a JCM patient. Although is a rare disease, it is a nature model o chronic hypercalcemia that can allows us new insights.

Nothing to Disclose: DFC, LO, SB, BBM, PHSC, RMM

Pub # P3-95

Session Information POSTER SESSION: CLINICAL - Parathyroid Hormone & Parathyroid Hormone-Related Peptide II (1:30 PM-3:30 PM)

Title Radiofrequency Ablation of Bone Metastasis Helps Control Hypercalcemia in Parathyroid Carcinoma

Author String JM Sharretts, WF Simonds
National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD

Body Background: Hypercalcemia is the major cause of morbidity and mortality in parathyroid carcinoma (PC). Surgical resection of metastases is the most effective means of reducing parathyroid hormone (PTH) and serum calcium levels. Successful radiofrequency ablation (RFA) of lung (1) and liver (2) metastases has also been reported. Adjuvant treatment with calcimimetics is limited by gastrointestinal side effects. FDG-PET has been used to localize PC not seen with sestamibi (3).
Clinical case: A 74 year-old man with PC, status-post 3 prior neck operations presented with increasing PTH and serum calcium levels. CT, MRI, US and sestamibi showed a 3 cm mass in the left neck. The mass was resected, but calcium and PTH levels remained unchanged. Pathology was consistent with PC. Subsequent FDG-PET/CT demonstrated 2 presumed metastatic lesions in the right iliac bone not seen on the sestamibi. Shortly thereafter, the patient presented with hypercalcemic crisis. Ionized calcium was 2.29 mmol/L (1.12-1.32) and creatinine was 2.93 mg/dL (0.77-1.19). He was treated with IV hydration and pamidronate. A foley catheter was required due to urinary retention. He was discharged but readmitted 3 days later due to severe hypercalcemia that again responded to hydration. He was not tolerating cinacalcet due to vomiting but was able to take 90 mg twice daily when it was given after ondansetron. Surgery was deferred due to his debilitated state, and instead he underwent RFA of the 2 iliac lesions. Pre-ablative ionized calcium (while on IV fluids) was 1.68 mmol/L and PTH was 1199.0 pg/mL. The patient tolerated the procedure well and was discharged 2 days later. He reported significant improvement in appetite, energy, and activity level. Calcium levels in the two subsequent months have ranged from 1.45-1.73 mmol/L, and PTH levels have varied from 699.0-1134.0 pg/mL with no obvious trend. The most recent serum creatinine was 1.99 mg/dL.
Conclusion: RFA of PC bone metastases has not been previously reported. Although the effect on calcium and PTH levels in this patient was modest, he has had a dramatic clinical improvement with minimal morbidity. RFA may be a useful palliative treatment even in patients with widely metastatic PC. FDG-PET may be a useful imaging modality in metastatic PC not localized with other studies. Ondansetron is beneficial in some patients who are unable to tolerate cinacalcet due to vomiting.

(1) Tochio M, et al., Cardiovasc Intervent Radiol 2010; 33:657
(2) Artinyan A, et al., J Clin Oncol 2008; 26:4039
(3) Gardner CJ, et al., J Clin Endocrinol Metab 2010; 95:4844

Nothing to Disclose: JMS, WFS

Pub # P3-96

Session Information POSTER SESSION: CLINICAL - Parathyroid Hormone & Parathyroid Hormone-Related Peptide II (1:30 PM-3:30 PM)

Title Safety of Vitamin D Replacement in Patients with Primary Hyperparathyroidism and Concomitant Vitamin D Deficiency

Author String D Wagner, R Hou
University of Rochester School of Medicine and Dentistry, Rochester, NY

Body **Background:** Patients with vitamin D deficiency and primary hyperparathyroidism (PHPT) often have higher PTH level, larger parathyroid adenoma and more fractures than hyperparathyroid patients with a normal vitamin D level. They are also more likely to develop hungry bone syndrome post surgery compared to those hyperparathyroid patients with normal vitamin D level. Common concerns are that replacing vitamin D in a patient with PHPT may raise serum and urine calcium level to unsafe levels and routine practice is not to treat the vitamin D deficiency.

Materials and Methods: We retrospectively collected data on 35 patients from our Endocrine clinic, age 22-89, who have been diagnosed with PHPT and vitamin D deficiency. These patients were treated with either 1000-2000 units vitamin D daily or 50,000 units Vitamin D weekly for 3-6 months. Data on serum calcium, 25-OH vitamin D, iPTH, PO₄, AlkPhos, 24 hr urinary calcium and creatinine, before and after the vitamin D replacement were collected. The charts were reviewed for evidence of nephrolithiasis, fractures and osteoporosis before and after treatment with vitamin D.

Results: 6/35 patients had Ca level increased 0.1-0.5 mg/dl, 5/35 between 0.5-1 mg/dl, none > 1mg/dl and 3 remained the same. Interestingly, 13/35 had Ca decreased 0.1-0.5 mg/dl, 4/35 decreased 0.5-1 mg/dl and 4/35 >1 mg/dl. Overall, the calcium and iPTH levels before and post treatment remained stable (P>0.05). The 25-OH vitamin D level is significantly higher after the treatment, as expected, P<0.001. All patients had the starting Ca level between 10.1-11.7 mg/dl. The highest post treatment Ca level was 12.6 mg/dl in a young patient with an initial Ca level of 11.7 mg/dl. Despite the high Ca, he developed no new symptoms and was found to have atypical adenoma at surgery. Creatinine remained stable in all patients. No new case of nephrolithiasis developed after vitamin D replacement.

Conclusion: Our retrospective analysis did not show any adverse outcome and demonstrates that replacing vitamin D is safe, effective, does not increase calcium to dangerous levels in patients with PHPT and usually decreases PTH level. We plan to develop a prospective study and follow Ca, Vit D, iPTH, PO₄, AlkPhos, creatinine, 24 hr urinary Ca at enrollment, 1 month, 6 months and 1 year, with gradual titration of vitamin level to achieve the lowest PTH level while maintaining an upper normal vitamin D level and lowest Ca possible.

(1) Grey A et al., JCEM 2005;90(4):2122-2126
(2) Tucci JR, European Journal of Endocrinology 2009;189-193

Nothing to Disclose: DW, RH

Pub # P3-97

Session Information POSTER SESSION: CLINICAL - Parathyroid Hormone & Parathyroid Hormone-Related Peptide II (1:30 PM-3:30 PM)

Title The Use of Cinacalcet Hydrochloride vs. Parathyroidectomy for Treatment of Secondary Hyperparathyroidism in Dialysis Patients Refractory to Alfacalcidol

Author String LV Egshatyan, LY Rozhinskaya, NS Kuznecov, EA Pigarova
FSI Endocrinologic Scientific Center, Moscow, Russian Federation

Body

BACKGROUND:
Disturbances of calcium (Ca) - phosphate (P) metabolism in chronic kidney disease (CKD) play an important role in bone disease. Decreased bone mass and disruption of microarchitecture occur early in the course of CKD and worsen with the progressive decline in renal function.
AIM: To determine the impact of cinacalcet hydrochloride and parathyroidectomy (PTx) on mineral disorder, bone turnover and bone mineral density (BMD) in haemodialysis patients with secondary hyperparathyroidism (sHPT).

MATERIALS AND METHODS: 50 patients were enrolled in this prospective study. All of them had surgical indications for PTx (intact parathyroid hormone (iPTH) (pg/ml) 1405.9±266, CaxP (mmol[^{sup}2]/l[^{sup}2]) 5.72±0.54 in the cinacalcet and iPTH 2297.7±281.2, CaxP 6.07±0.25 in the PTx groups). 40% of the patients underwent PTx, 60% of them were treated with cinacalcet 30-180 mg/day. IPTH, CaxP, BMD by dual x-ray absorptiometry, osteocalcin (OC, ng/ml) and B-crosslaps (CTx, ng/ml) were examined at baseline and 6 months.

RESULTS: At baseline:
Cinacalcet group: L2-L4: T-score -1.63±0.29 (n=16); Z-score -0.9±0.4 (n=14). Rad 33%: T-score -1.98±0.16 (n=16); Z-score -1.78±0.32 (n=14). Total femur: T-score -1.75±0.25 (n=16); Z-score -0.7±0.3 (n=14). CTx 4.26±0.55; OC 294.1±13.34
PTx group: L2-L4: T-score -1.81±0.38 (n=12); Z-score -0.9±0.4 (n=8). Rad 33%: T-score -3.2±0.5 (n=12); Z-score -3.99±0.52 (n=8). Total femur: T-score -1.51±0.25 (n=12); Z-score -1.43±0.41 (n=8). CTx 4.83±0.25 OC 283.2±9.67.

In 6 months mean iPTH and Ca[times]P levels decreased by 56.7% (p<0.01) and 36.2% (p<0.01) in the cinacalcet group vs. 90.7% (p<0.01) and 55.5% (p<0.01) in the PTx group.
Dynamics BMD from baseline in the cinacalcet vs. PTx groups: L2-L4 +0.07±0.86% (p>0.05) vs. +6.83±2.9% (p<0.01); Rad 33%: -0.16±0.99% (p>0.05) vs. +11.4±8.4% (p<0.01); total femur +1.82±0.97% (p>0.05) vs. +12.37±2.3% (p<0.01).

At 6 months in the both cinacalcet and PTx groups CTx decreased by 21.4% (p<0.01) vs. 58.7% (p<0.01) and OC 1.4% (p<0.01) vs. 26.9% (p<0.01). CONCLUSIONS: PTx and cinacalcet therapy was associated with significant reductions in iPTH, Ca and CaxP, but bone resorption and formation markers decreased better in the PTx group compared to cinacalcet group. At 6 months active restoration of BMD was found in the PTx patients, and patients treated with cinacalcet showed stabilization of BMD.

Nothing to Disclose: LVE, LYR, NSK, EAP

Pub # P3-98

Session Information POSTER SESSION: CLINICAL - Parathyroid Hormone & Parathyroid Hormone-Related Peptide II (1:30 PM-3:30 PM)

Title Preoperative Parathyroid Venous Sampling in Patients with Non-Localizing Primary Hyperparathyroidism

Author String JR Mindel, AC Venbrux, SM Knoll, JL Cohen
George Washington University, Washington, DC; George Washington University, Washington, DC; George Washington University, Washington, DC

Body

Background: The current practice of pre-operative parathyroid imaging with ultrasound and sestamibi scans in patients with primary hyperparathyroidism (PHP) allows for minimally invasive surgery targeted selectively to the overproducing gland. However, if pre-operative imaging fails to localize an adenoma, open, bilateral surgical exploration may be required. The purpose of this study was to evaluate the utility of highly selective parathyroid venous sampling (PVS) with rapid parathyroid hormone (PTH) assay for the localization of parathyroid adenomas in patients with PHP who had indeterminate results on pre-operative imaging.

Methods: Clinical and laboratory data from 16 patients with non-localizing findings on ultrasound and sestamibi were reviewed. All 16 patients underwent pre-operative PVS with rapid PTH assays done in real-time during the sampling procedure. The internal jugular (IJ) veins were sampled initially in all subjects. Additionally, smaller tributary veins were sampled bilaterally using 1 mL steerable microcatheters. Operative notes and pathology reports were reviewed to confirm the diagnosis and intraoperative location of the parathyroid lesion.

Results: PVS provided accurate lateralization in 14 of 16 (87.5%) patients, all of whom were felt to have a single adenoma. The remaining 2 patients had equivalent PTH values bilaterally, as well as symmetrically equivalent imaging, and were felt to have four-gland hyperplasia. When the IJ PTH levels were considered alone, 5 of the 14 patients (36%) did not show significant lateralization. Only after evaluating PTH values from the smaller tributary veins was significant lateralization appreciated in these patients. 10 of the 14 patients with lateralization on PVS have undergone surgery; in all 10 patients (100%), PVS correctly predicted which side of the neck the parathyroid adenoma was located.

Discussion: Minimally invasive parathyroidectomy is preferred due to shorter time, lower cost, and less morbidity. In our sample, 87.5% of patients with PHP and negative pre-operative imaging had successful localization with PVS enabling minimally invasive surgery. The accuracy of PVS depends upon a skilled operator, and is improved with increased sampling of smaller tributary veins when compared to IJ sampling alone. The disadvantage of PVS is that it is time consuming and expensive. These disadvantages may be offset by an increased surgical success rate that avoids the need for a second surgery.

Nothing to Disclose: JRM, ACV, SMK, JLC

Pub # P3-99

Session Information POSTER SESSION: CLINICAL - Parathyroid Hormone & Parathyroid Hormone-Related Peptide II (1:30 PM-3:30 PM)

Title Phosphate Levels Inversely Correlate with Subclinical Atherosclerosis in Patients with Primary Hyperparathyroidism

Author String F Athanasouli, L Papadopoulos, A Vemmou, K Chalaris, E Efthimiou, C Papamichael, K Stamatelopoulos, E Terpos, M Alevizaki
Athens University School of Medicine, Athens, Greece; Athens University School of Medicine, Athens, Greece

Body Introduction. Although data on the association of primary hyperparathyroidism (pHPT) with cardiovascular disease are accumulating, there is still controversy on this issue. In particular it is not clear whether parathyroid hormone (PTH) per se or other disease-related molecules such as calcium, phosphate or vitamin D3 correlate with accelerated subclinical or overt atherosclerosis.
Methods. Seventy patients with pHPT were consecutively recruited from the outpatients clinic of an academic department of endocrinology. In all patients, arterial stiffness was assessed by measurement of pulse wave velocity (PWV) between carotid and femoral arteries while reflected waves and central blood pressures were evaluated by pulse wave analysis. Carotid intima-media thickness (IMT) and the presence of plaques in the carotid and common femoral arteries were assessed by high-resolution ultrasonography.
Results. Phosphate levels but not calcium, PTH or vitamin D3 levels inversely correlated with IMT ($r=-0.247$, $p=0.042$) and PWV (-0.244 , $p=0.046$). Patients with PTH (>163 ng/l) or calcium (>11.2 mg/dl) in the highest tertile presented significantly higher prevalence ($p=0.023$) of atherosclerotic plaques (11/23, 49%) as compared to the remaining patients (10/47, 21%). By multivariate analysis, among other risk factors, phosphate levels independently determined PWV ($p=0.032$).
Conclusions. In patients with pHPT, PTH, calcium and phosphate levels correlate with subclinical atherosclerosis. However, only phosphate levels independently correlated with PWV, a marker of arterial stiffening. Further research is needed to assess the possible role of phosphate levels on atherosclerosis in pHPT.

Nothing to Disclose: FA, LP, AV, KC, EE, CP, KS, ET, MA

Pub # P3-100

Session Information POSTER SESSION: CLINICAL - Parathyroid Hormone & Parathyroid Hormone-Related Peptide II (1:30 PM-3:30 PM)

Title Hypermagnesemia-Induced Hypocalcemia and the Effects on Parathyroid Hormone: Case Report and Literature Review

Author String B Alfonso, T Araki, N Needleman, A Busta
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Body

Introduction:
Continuous intravenous magnesium (Mg) infusion is frequently used in pregnancy for tocolysis or seizure prophylaxis. However, endocrinological complications are not well described.

Case presentation:
A 30 year old woman presented at 24 weeks gestation with pre-eclampsia, hemolysis, elevated liver enzymes and low platelets (HELLP syndrome). Intravenous magnesium sulfate (MgSO₄) was initiated immediately with a 6g loading dose, followed by a 2 g/hour infusion. The serum Mg reached high levels of 7.3 mg/dl at 12 hours. The parathyroid hormone (iPTH) level was low at the start of the Mg infusion, and remained low (6.7 -13.2 pg/ml range) in the first 12 hours; the calcium (Ca) levels decreased from 8.8 to 5.6mg/dL over the same 12 hours. After 18 hours, the MgSO₄ infusion rate was decreased, and the iPTH levels rose (72-149 pg/ml range). The Ca continued to decrease over the next day, reaching a nadir of 4.8 mg/dL at 24 hours. The Mg infusion was stopped at 28 hours, and the iPTH level continued to rise, up to 188 pg/mL at 34 hours. Thereafter the serum Ca, Mg and the iPTH levels normalized. Remarkably, the patient was asymptomatic through hypermagnesemia, hypocalcemia and with low iPTH. She underwent an emergent cesarean section and was discharged after 4 days.

Discussion:
MgSO₄ infusion is an established treatment for obstetrical emergencies. Its effects on calcium/parathyroid physiology and the exact mechanism remains unclear (1,2,3)
One theory postulates that Mg could inhibit PTH secretion the same way that Ca does, given that both Mg and Ca are divalent cations (5). Another mechanism claims that the hypermagnesemia may inhibit renal Ca reabsorption in the loop of Henle (4). In our case we documented fluctuating levels of iPTH and Mg. We believe that the variations of iPTH may be due to the PTH's short half-life and to the rapid response of PTH secretion to MgSO₄ infusion dose changes and discontinuation. We think that initially the increased Mg level can cause inhibition of renal Ca reabsorption (4); then, given its divalent nature, Mg is able to inhibit iPTH secretion. This order of events has been noted whether starting from baseline normal Mg and Ca (4) or low Ca and Mg (5). Further investigation of Mg and PTH interactions will need to be done under a constant Mg infusion in order to elucidate the mechanism of interaction.

1. Cholst IN et al., N Engl J Med 1984; 310:1221-1225.
2. Mayan H et al., Nephrol Dial Transplant. 1999; 14:1764-1766.
3. Koontz SL et al., Am J Obstet Gynecol. 2004; 190:1773-1776.
4. Cruikshank DP et al., Obstet Gynecol. 1981; 58:430-434.
5. Wiegmann T et al., Arch Intern Med. 1977; 137(7):953-955.

Nothing to Disclose: BA, TA, NN, AB

Pub # P3-101

Session Information POSTER SESSION: CLINICAL - Parathyroid Hormone & Parathyroid Hormone-Related Peptide II (1:30 PM-3:30 PM)

Title Hyperparathyroidism in Pregnancy from Intrathyroidal Parathyroid Adenoma Successfully Managed Operatively during Third Trimester

Author String CT Luk, DS Feig
University of Toronto, Toronto, Canada

Body **Introduction:** Twenty-five percent of cases of hyperparathyroidism are diagnosed during the childbearing years (1). In pregnancy, hyperparathyroidism may be asymptomatic, but is associated with significant adverse outcomes for both mother and child, and may warrant consideration of surgical management, even if recognized beyond the second trimester. We describe an example of this approach which was which was further complicated by the rare finding of an intrathyroidal parathyroid adenoma.

Clinical Case: A 39-year-old G2 P1 woman was 28 weeks gestation when undergoing follow-up for to a history of IgA nephropathy. She was found to have persistently elevated ionized calcium, 6.2-6.4 mg/dL (4.6-5.4). She was asymptomatic, with normal renal function, creatinine 0.62 mg/dL (0.6-1.2), estimated GFR > 90 mL/min, but PTH was significantly elevated at 14.4 ng/L (1.6-6.9). She was referred for urgent neck ultrasound and MRI, which did not show evidence of a parathyroid adenoma, but did identify a left 1.3 cm thyroid nodule.

At 30 weeks gestation, she underwent surgical neck exploration. Three parathyroid glands were identified as normal during surgery, but a fourth could not be found. A left hemi-thyroidectomy was performed for the apparent nodule, which unexpectedly was identified as hyperplastic parathyroid tissue, proving to be a rare, intrathyroidal parathyroid adenoma. Because her post-operative PTH level was 1.1 ng/mL (<1.1 ng/mL 1 hour postoperatively predicts hypocalcemia (2)), she was started immediately on calcitriol 0.25 mcg po bid and calcium carbonate 1 g (elemental) po tid. Her calcium remained normal postoperatively, and her course was otherwise uncomplicated. She delivered a healthy baby at term with no postdelivery development of hypocalcemia.

Conclusion: While hyperparathyroidism in pregnancy remains rare, and may be discovered incidentally, this does not mitigate the significant risk of complications of maternal hypercalcemia which include 48% incidence of fetal loss (3), and neonatal tetany. Treatment of choice is parathyroidectomy in the second trimester, but the few cases of surgery after 27 weeks gestation that have been reported to date, have found few complications (4). This case also illustrates successful resolution and surgical management despite a relatively late diagnosis. Additionally, as we have seen here, when evaluating thyroid nodules, the potential for ectopic parathyroid tissue should not be forgotten.

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(2) Vescan A, Witterick I, Freeman J. Laryngoscope 2005; 115(12):2105-8
(3) Norman J, Politz D, Politz P. Clin Endocrinol 2009; 71(1):104-9
(4) Schnatz PF, Thaxton S. Obstet Gynecol Surv 2005; 60(10):672-82

Nothing to Disclose: CTL, DSF

Pub # P3-102

Session Information POSTER SESSION: CLINICAL - Parathyroid Hormone & Parathyroid Hormone-Related Peptide II (1:30 PM-3:30 PM)

Title Parathyroid Hormone Is Associated with Biomarkers of Chronic Inflammation, Independent of Vitamin D Status, in Obese Adolescents

Author String R Alemzadeh, J Kichler
Medical College of Wisconsin, Milwaukee, WI; Children's Hospital of Wisconsin, Milwaukee, WI

Body **Background/Aims:** Parathyroid hormone (PTH) and vitamin D interactively regulate calcium homeostasis, and thereby modulate insulin sensitivity and blood pressure in adults. In addition, PTH has been shown to correlate with several markers of metabolic syndrome (MBS) within a normocalcemic adult population. Hypovitaminosis D (25-OH-vit D<20 ng/mL) and resultant hyperparathyroidism is among endocrine derangements of obesity. We hypothesize that circulating PTH, but not vitamin D, might be associated with indices of MBS.

Methods: we reviewed anthropometric, body composition and biochemical data including 25-OH-vit D, and PTH from 133 obese adolescents (78 F/55 M) aged 14.8 ± 1.4 years with body mass index (BMI) >95th% (38.6 ± 9.1 kg/m²). Serum levels of 25-OH-vit D and PTH were analyzed in relationship with ethnicity/race [Caucasian (C), Hispanic (H), and African American (AA)], fat mass (FM), high sensitivity c-reactive protein (hs-CRP), HbA1c, lipid profile, fasting glucose, insulin and the homeostatic model assessment for insulin resistance (HOMA-IR).

Results: Hypovitaminosis D was present in 45.1% of all patients, but was more prevalent in H (56.4%; $p<0.001$) and AA (63.9%; $p<0.001$) subgroups than in C subgroup (25.9%). Serum 25-OH-vit D and PTH were inversely correlated ($r=-0.75$; $p<0.0001$) for the entire cohort ($p<0.001$). While FM was negatively correlated with 25-OH-vit D ($r=-0.295$; $p<0.001$), it was positively correlated with PTH levels ($r=0.38$; $p<0.0001$). However, AA subgroup displayed higher PTH/25-OH-vit D than H and C subgroups ($p<0.05$). Further, 57.9% of cohort met diagnostic criteria for MBS displaying higher BMI, PTH, HOMA-IR, hs-CRP, triglycerides (TG), TG/HDL-C and HbA1c, but lower 25-OH vit D than subjects without MBS ($p<0.02$). However, there was no difference in prevalence of hypovitaminosis D between MBS and non-MBS groups (53.2% vs 32.9%; $p=0.66$). PTH was positively correlated with hs-CRP ($r=0.18$; $p<0.05$) and TG/HDL-C ($r=0.22$; $p<0.025$), whereas PTH and 25-OH-vit D were negatively correlated with HbA1c ($r=-0.18$ & $r=-0.19$ $p<0.05$) when controlled for FM.

Conclusions: Serum PTH level, but not 25-OH-vit D, is an independent predictor of chronic inflammation. Elevated serum PTH may be associated with increased risk of cardiovascular morbidity in obese adolescents. Further, African American adolescents have an increase in serum PTH at a lower 25-OH-vit D level than other ethnic groups implying negative cardiometabolic risks of higher PTH levels.

Nothing to Disclose: RA, JK

Pub # P3-103

Session Information POSTER SESSION: CLINICAL - Parathyroid Hormone & Parathyroid Hormone-Related Peptide II (1:30 PM-3:30 PM)

Title Severe Hypercalcemia in a Patient with Liver Metastasis of Unknown Primary: An Unusual Case of Coexistent Primary Hyperparathyroidism (PHPT) and Humoral Hypercalcemia of Malignancy (HHM)

Author String HN Abou Assi, TJ Weber, JM Perkins
Duke University Medical Center, Durham, NC

Body **Background:** Hypercalcemia is one of the most common metabolic abnormalities. PHPT and HHM are the 2 most common causes. An intact PTH (iPTH) level is the classic discriminator between PHPT and parathyroid independent hypercalcemia. Herein, we describe a case of severe hypercalcemia in a patient with liver metastasis.

Clinical Case: Our patient is a 64 year-old man with history of CAD, gout and nephrolithiasis in the past. Two months prior to presentation he developed nausea, poor appetite, abdominal pain and weight loss. He was seen by GI and colonoscopy was normal. Abdominal CT showed innumerable liver lesions concerning for metastasis. CT guided biopsy of the liver lesions was done on 12/15/10. He presented to an outside facility on 12/17/10 with altered mental status, anorexia and abdominal pain. On presentation his Calcium (Ca) level was 15.6 mg/dl (8.4-10.2), Albumin (alb): 3.4 g/dl (3.5-5.2), Ionized Ca(ICa): 8.9 mg/dl (4.6-5.4), Phosphorus (Ph): 4.2 mg/dl (2.5-5.0), Creatinine (Cr): 2.21 mg/dl (0.61-1.24), LFTs mildly elevated, amylase and lipase significantly elevated. He received IV fluids and was transferred to our facility the next day. On 12/18/10 his Ca was 13.7 mg/dl (8.7-10.2), Alb: 2.6 g/dl (3.5-4.8), Ph: 4.3 mg/dl (2.3-4.5), ICa: 1.98 mmol/L (1.15-1.32), Cr: 2.5 mg/dl (0.6-1.3). He received IV fluids, and with HHM as the working diagnosis, he was treated with 4 mg IV Zoledronic acid and 4 doses of Calcitonin. He showed rapid clinical improvement and his Ca levels normalized over the next 4 days. Further work up showed elevated iPTH: 129 pg/ml (14-72), TSH and 25 (OH)vitaminD were normal, 1,25 Di(OH) vitamin D was low, SPEP and UPEP were unremarkable. Endocrinology consult was called. The severity of the hypercalcemia raised the concern for more than one mechanism, hence PTHrp was checked and was elevated at 11 pmol/L (<2.0). Pathology report showed poorly differentiated non small cell carcinoma, the primary is still unknown. The patient was discharged on 12/23/10 in a stable condition with normal Ca levels, to follow up with Oncology and Endocrinology.

Conclusion: PHPT is not uncommon in patients with cancer, however the coexistence of PHPT and HHM is much less likely. Review of the literature revealed only few case reports. Together with our patient, these cases emphasize the importance of considering a broad differential in the work up of patients with hypercalcemia.

Nothing to Disclose: HNAA, TJW, JMP

Pub # P3-104

Session Information POSTER SESSION: CLINICAL - Parathyroid Hormone & Parathyroid Hormone-Related Peptide II (1:30 PM-3:30 PM)

Title A New Deletion of PTHLH Associated with Brachydactyly Type E and Breast Hypoplasia

Author String C Thomas-Teinturier, C Silve, P Bougneres, A Linglart
Saint Vincent de Paul Hospital, Paris, France; INSERM, Paris, France; Bichat Hospital, Paris, France

Body Autosomal-dominant brachydactyly type E (BDE) is a congenital limb malformation characterized by small hands and feet as a result of shortened metacarpals and metatarsals. PTHRP is known to regulate the balance between chondrocyte proliferation and the onset of hypertrophic differentiation during endochondral bone development. Deletions in PTHLH, the gene coding for parathyroid hormone related protein (PTHRP) have been identified as a cause of BDE in 5 families (1, 2) and inactivation in PTHRP in mice results in short-limbed dwarfism and abnormalities in tooth and breast development (3).
We report a mother and daughter with short stature, brachydactyly and breast hypoplasia in the daughter. The daughter AD was born with a normal height 49 cm, growth curve shows normal growth velocity, her height is 145 cm at 12yr and 9 months (-1 SD). She has shortening of IV and V th metacarpal, forearms and lower legs. No tooth was lacking but she had many dental malpositions. She has pubic hair P4, axillary hair but no breast development. Ultra sound of the pelvic shows a uterine length of 54 mm with thickening of endometrium and serum estradiol levels of 162 pmol/l corresponding to pubertal stage III. Bone x-rays show low bone mineral density.
The mother's height is 147 cm. She has shortening of III, IV and Vth metacarpal, III and IVth metatarsal, forearms and lower legs. She has small breasts but could breastfeeding her children. Bone x-rays show low bone mineral density.
No mutation in the GNAS1 gene was detected. We identified a heterozygous deletion c.47_101+73del128 in PTHLH in the mother and daughter. The deletion is expected to result in a non-secreted truncated protein in which the last 18 amino-acids of the signal peptide are replaced by an unrelated 27 amino-acid sequence. In conclusion, we identified a new deletion in PTHLH in a family with brachydactyly type E and report the first case associated with breast hypoplasia.

(1) Klopocki E et al., Am J Hum Genet 2010;86:434

(2) Maass PG et al., Hum Mol Genet 2010; 19:848

(3)Wysolmerski JJ et al., Development 1998; 125:1285

Nothing to Disclose: CT-T, CS, PB, AL

Pub # P3-105

Session Information POSTER SESSION: CLINICAL - Parathyroid Hormone & Parathyroid Hormone-Related Peptide II (1:30 PM-3:30 PM)

Title Normocalcemic Hyperparathyroidism: Studies on Bone Loss over a Ten-Year Follow-up Time

Author String R Antonelli, S Falcone, A Scillitani, AS Salcuni, V Carnevale, C Battista, E Romagnoli, S Minisola, I Chiodini, M Passeri, M Calanchini, A Fabbri
Osp S Eugenio & Osp CTO, Universit[agrave] Tor Vergata, Roma, Italy; Osp Casa Sollievo della Sofferenza, San Giovanni Rotondo, Italy; Policlinico Umberto I, Roma, Italy; Policlinico Universitario Milano, Milano, Italy

Body Among the 1500 admissions/year for ten years referred to our outpatient endocrine clinics, we collected 471 subjects with high serum levels of PTH. A diagnosis of primary hyperparathyroidism or secondary hyperparathyroidism due to renal failure was performed in 123 and 94 respectively. Other 219 subjects had a reduction in the normal range of PTH after correction of the vitamin D insufficiency. The remaining 35 subjects had serum calcium and creatinine in the normal range and also after correction of vitamin D insufficiency had a high PTH level. Among them 10 dropped out and the other 25 were followed up for 1-10 years. In these 25 hyperparathyroid subjects we measured at baseline and over time serum calcium, creatinine 25OH vitamin D, PTH, urinary calcium, bone mineral density, AP and Lateral vertebral X ray (to evaluate morphometric vertebral fractures) and renal ultrasonography. Over time 10 out of the 25 hyperparathyroid subjects became hypercalcemic (group A, followed up for 4-10 years) while the others continued to be normocalcemic (group B, followed up for 1-10 years). The two groups were not different at baseline for calcium [M±SD (9.6 mg/dL ± 0.5 vs 9.5 ± 0.5, A vs B)], creatinine (0.7 mg/dL± 0.2 vs 0.7± 0.3, A vs B), BMD expressed as T-Score at lumbar spine(LS) (-2.4 vs -2.0, A vs B) and femoral neck (FN) (-2.1 vs -1.8, A vs B) while subjects in group A were significantly older than in group B (70 years ± 9 vs 65 ± 7; p < 0.05). In group A (Normocalcemic hyperparathyroid group) followed over time, 9 patients had a significant reduction of BMD, 3 also a fracture (2 vertebral and 1 Colles) and one also a kidney stone; three patients were operated on and a parathyroid adenoma was removed. In group B (not primary/not secondary hyperparathyroid group) followed over time, 3 subjects had a significant reduction of BMD. The BMD at the last visit was at LS -2.9 Tscore vs -2.1 (A vs B) and at FN -2.4 Tscore vs -2.0 (A vs B). In conclusion our retrospective longitudinal study suggest that patients with normocalcemic hyperparathyroidism have overtime an important involvement of bone (reduction of BMD and incidence of fractures) which is also higher than in not primary/not secondary hyperparathyroid patients.

Nothing to Disclose: RA, SF, AS, ASS, VC, CB, ER, SM, IC, MP, MC, AF

Pub # P3-106

Session Information POSTER SESSION: CLINICAL - Parathyroid Hormone & Parathyroid Hormone-Related Peptide II (1:30 PM-3:30 PM)

Title Vitamin D Status in Primary Hyperparathyroidism

Author String A Scillitani, C Battista, S Minisola, E Romagnoli, M Mascia, I Chiodini, V Carnevale, V Frusciante, DEC Cole
Casa Sollievo della Sofferenza Hospital, IRCCS, San Giovanni Rotondo, Italy; University of Roma [ldquo] Sapienza[rdquo], Roma, Italy; Fondazione Policlinico IRCCS, University of Milan, Milan, Italy; Casa Sollievo della Sofferenza Hospital, IRCCS, San Giovanni Rotondo, Italy; Casa Sollievo della Sofferenza Hospital, IRCCS, San Giovanni Rotondo, Italy; University of Toronto, Toronto, Canada

Body Hypovitaminosis D is common in patients with Primary Hyperparathyroidism (PHPT) and several mechanisms could theoretically predispose to vitamin D deficiency. Indeed the excess PTH induces an accelerated catabolism of 25 hydroxy-vitamin D (25OHD) by activating both renal 1 α -hydroxylase and liver 24 hydroxylase.
We evaluated vitamin D serum levels in PHPT patients and compared them with 2 different groups of healthy family members: those sharing the same environment only--that are, in-law relatives (ILR) or those sharing genetic background and environment--namely, first degree relatives (FDR).
This study is part of a larger one in which an accurate evaluation of PHPT patients with matched FDR and ILR subjects is performed. Eighty-five PHPT subjects of 400 patients were initially collected because we had at least one FDR and one ILR subject (trios) for each patient. Subsequently 36 patients were excluded since they had reported taking vitamin D supplementations.
Finally we analyzed data on 49 families. Mean age \pm SD of PHPT patients, FDR and ILR subjects was 59.6 \pm 9.9, 43.4 \pm 14.5 and 51.8 \pm 14.7 years, respectively. Serum 25OHD levels were 14.1 \pm 7.5, 21.5 \pm 11.4, 22.2 \pm 13.9 ng/ml, respectively and were significantly different between patients and either group of healthy subjects (p< 0.05).
Univariate analysis of variance by GLM was utilized in order to correct for other covariates (season, age). Serum 25OHD was significantly lower in PHPT patients in respect the two groups of healthy subjects after adjustment for these covariates.
Our study confirms that, PHPT patients have lower 25OHvitamin D levels than healthy subjects sharing the same environment and a similar genetic background.

Nothing to Disclose: AS, CB, SM, ER, MM, IC, VC, VF, DECC

Pub # P3-107

Session Information POSTER SESSION: CLINICAL - Parathyroid Hormone & Parathyroid Hormone-Related Peptide II (1:30 PM-3:30 PM)

Title Use of Teriparatide for Symptomatic Hypoparathyroidism

Author String M Jung, H Karanchi, AR Sadhu, D Hamilton
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Body

Introduction:
Acute hypocalcemia is a medical emergency. Sporadic hypoparathyroidism is rare in adults but can result in severe hypocalcemia. The [ldquo]hungry-bone syndrome[rdquo] results from pre-surgical hyperparathyroidism and contributes to hypocalcemia as well. We present two representative cases successfully treated with subcutaneous (s.c.) synthetic human PTH(1-34) [teriparatide] following failure with conventional treatment.

Case Presentation:

1st case: A 93 year old woman with hypertension and dementia presented with altered mental status. She had no history of neck surgery. She had positive Trousseau's sign. Laboratory data was remarkable for total serum calcium - 3.6 mg/dL (nl. 8.6-10.6) and ionized calcium - 0.47 mmol/L (nl. 1.17-1.29), phosphorus - 6.3 mg/dL (nl. 2.5-4.8), PTH - 6 pg/mL (nl. 13-65), 25-OH vitamin D -11 ng/mL (nl. 30-80). Severe symptomatic hypocalcemia persisted besides treatment with multiple doses of oral and IV calcium, oral cholecalciferol and calcitriol. She also refused to take oral medicines frequently due to lack of insight.

2nd case: A 44 year old woman with hypertension, ESRD and tertiary hyperparathyroidism treated with four gland parathyroidectomy and implantation of 50% of one gland in the left forearm 10 days prior to presentation. She presented with numbness and tingling, perioral and in extremities and myalgias. She had positive Chvostek's and Trousseau's sign. Laboratory data was significant for total serum calcium - 6.6 mg/dL ionized calcium - 0.77 mmol/L, phosphorus - 2.2 mg/dL, PTH - 5 pg/mL, 25-OH vitamin D - 14 ng/mL. In each case, after several days of unsuccessful vigorous calcium and vitamin D therapy, a trial of teriparatide 20 [micro]g s.c. (twice daily in the 1st case and once daily in the 2nd case) resulted in gradual normalization and stabilization of serum calcium and symptomatic improvement. In the 1st case, a significant decrease in transtubular phosphate reabsorption was documented within six hours of the first dose.

Discussion:
Teriparatide subcutaneous injection provides rapid correction of calcium and phosphate balance in selected patients where extenuating circumstances prevent more conventional treatment with calcium and vitamin D. In the 1st case, the patient's mental status prevented oral treatment; in the 2nd case, the presence of ESRD and transient high skeletal uptake (hungry bone syndrome) responded to this treatment.

Nothing to Disclose: MJ, HK, ARS, DH

Pub # P3-108

Session Information POSTER SESSION: CLINICAL - Parathyroid Hormone & Parathyroid Hormone-Related Peptide II (1:30 PM-3:30 PM)

Title Lithium Carbonate Use May Enhance Hypercalcemia in a Patient with Primary Hyperparathyroidism

Author String DT Huberman, M Blum
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Body Case presentation: This 70 year old woman who has been taking Lithium Carbonate (Li) for several years for bipolar disorder had been normocalcemic 2 years ago. There was recent, severely altered sensorium associated with hypercalcemia of 13.5mg/dL (Ca) and high iPTH(152pg/mL). The hypercalcemia was refractory to copious hydration (4 L water/day). Other laboratory assessment was unremarkable. After transfer to NYU Langone Medical Center a 1.3cm parathyroid adenoma (PA) was resected. Intra-operatively the iPTH fell from 117.6 to 24.8pg/mL but Ca remained 10.2 mg/dL for 2 days, and has remained normal since. The patient's psychiatrist felt that cessation of Li would be extremely ill advised, so the medication was continued. Discussion: There are 2 main mechanisms by which Li causes hypercalcemia: 1)Li enhances PTH secretion by decreasing parathyroid gland sensitivity to calcium. 2)Li increases urinary calcium reabsorption and decreases urinary calcium excretion. Reports have shown in Li treated patients an increase in mean parathyroid gland volume, four gland hyperplasia, and PA. It is not known whether Li use leads to the formation of PA, or unmasks asymptomatic, undetected PA. Nephrogenic diabetes insipidus is the most common renal side effect associated with chronic Li use. It is likely that Li accumulates in the collecting tubules and then interferes with the action of anti diuretic hormone. The resultant polyuria in a patient with Li induced nephrogenic diabetes insipidus may further exacerbate a patient's hypercalcemia. Li-induced polyuria is associated with decreased renal medullary sodium and osmolality, and defective renal urinary concentrating ability, which may have contributed to hypercalcemia that was refractory to aggressive hydration in our patient. Hence, hydration with sodium-rich fluid might have been beneficial. Conclusions: We report a case of significant hypercalcemia that was resistant to copious hydration and lagged behind adenoma resection in a patient with primary hyperparathyroidism (HPT) and chronic Li use. Lessons include the need for caution in the use of Li in a HPT patient with bipolar disorder, consideration of discontinuing Li when a HPT patient has an unusually high calcium level, and attention to the use of sodium-rich hydration to manage hypercalcemia.

Nothing to Disclose: DTH, MB

Pub # P3-109

Session Information POSTER SESSION: CLINICAL - Parathyroid Hormone & Parathyroid Hormone-Related Peptide II (1:30 PM-3:30 PM)

Title Is It Benign Familial Hypocalciuric Hypercalcemia or Primary Hyperparathyroidism?

Author String S Thukuntla, S Sequeira, P Kumar
University of Texas Southwestern Austin Program, Austin, TX; University of Texas Southwestern Austin Program, Austin, TX

Body

Background:
Although PTH-dependent hypercalcemia is commonly due to primary hyperparathyroidism (PHPT), it is important to exclude benign familial hypocalciuric hypercalcemia (BFHH) since surgery may cure PHPT patients but is not required in BFHH.

Clinical Case:
A 20 year-old female presented with abdominal pain and nausea for two days prior to admission. Her corrected serum calcium was 11.6 (8.5-10.5 mg/dL), phosphorus was 3.3 (2.5-4.6 mg/dL), magnesium was 1.1 (1.7-2.8 mg/dL) and iPTH level was 85(12-88 pg/mL) which were confirmed on repeat testing. There was no history of renal insufficiency or kidney stones. She was adopted and did not know her family history. She had never taken lithium or thiazide diuretics. 24-hour urine calcium was 98 mg/d, ratio of calcium clearance to creatinine clearance was <0.01 and 25(OH) vitamin D level was 26 (15-75 pg/mL). Review of records from a prior hospital admission two years ago also revealed hypercalcemia. A sestamibi scan and a parathyroid ultrasound were unable to localize a parathyroid adenoma or hyperplasia. There were no stigmata of MEN syndromes and testing for them was negative as well. DNA analysis of the calcium sensing receptor mutation (CaSR) showed functionally significant heterozygous mutation of the CaSR on exon 7 consistent with R990G polymorphism.

Discussion:
The R990G mutation is a benign polymorphism of CaSR gene found in about 3% of the population. The Arg990Gly SNP (R990G, base change 2968A>G) produces an activating gain-of-function mutation of the CaSR gene and predisposes to hypercalciuria and nephrolithiasis. On the contrary, BFHH is an autosomal dominant condition due to inactivating loss-of-function mutation in the CaSR gene. Thus, the body is unable to sense hypercalcemia, PTH secretion is not inhibited and there is tubular absorption of calcium and resultant hypocalciuria.

Conclusion:
Our patient's clinical history, young age, hypercalcemia, hypocalciuria suggests benign familial hypocalciuric hypercalcemia but the over activity of the CaSR makes it unlikely. Differential diagnosis includes primary hyperparathyroidism with an added factor causing hypocalciuria. Since only the protein coding part of the CaSR is examined in the mutation testing, she may have an inactivating mutation in the noncoding region which was not included in DNA testing. The patient was not referred to surgery for bilateral neck exploration since we are unclear about the diagnosis.

1. Varghese, J et al. Benign Familial Hypocalciuric Hypercalcemia. Endo Pract. 2010, Dec 6:1-16.
2. Vezzoli, G et al. R990G polymorphism of calcium-sensing receptor does produce a gain-of-function and predispose to primary hypercalciuria. Kidney Int 2007 Jun; 71(11):1155-1162

Nothing to Disclose: ST, SS, PK

Pub # P3-110

Session Information POSTER SESSION: CLINICAL - Osteoporosis, Fractures & Bone Density (1:30 PM-3:30 PM)

Title Brown Adipose Tissue Is Positively Associated with BMD and Inversely Associated with VAT in Young Non-Obese Women

Author String MA Bredella, PK Fazeli, LM Freedman, G Calder, W Cai, AH Singh, CJ Rosen, A Klibanski
Massachusetts General Hospital, Boston, MA; Massachusetts General Hospital, Boston, MA; Maine Medical Center Research Institute, Scarborough, ME

Body Anorexia nervosa (AN) is associated with severe depletion of body fat and significant loss of bone mineral density (BMD). Bone and fat cells arise from a common mesenchymal precursor, capable of differentiation into osteoblasts and adipocytes. Prior studies have focused on brown adipose tissue (BAT) and its relationships to other fat depots, BMD and adaptive thermogenesis. BAT is lower in obese individuals and decreases during aging. Bone morphogenetic protein 7 (BMP-7) has recently been identified as an important promoter of BAT differentiation, suggesting a possible positive effect of BAT on BMD. AN is associated with marked decreases in visceral adipose tissue (VAT) and the identification of BAT and its relationship with BMD is unknown. We studied 15 women, 5 with AN (mean age 30 ± 6.3 years), 5 recovered AN (AN-R) and 5 healthy non-obese controls (HC) of comparable age. BMI of the AN group ($18.3 \pm 0.9 \text{ kg/m}^2$) was lower than that of AN-R ($22.4 \pm 3.8 \text{ kg/m}^2$) and HC ($21.9 \pm 14 \text{ kg/m}^2$) ($p=0.04$). All women underwent whole body FDG-PET/CT for quantification of BAT using a standard cooling protocol, DXA for assessment of total body, spine, hip BMD, fat and lean mass, and single slice MRI at L4 for assessment of abdominal fat compartments. Pref-1, a regulator of osteoblast and adipocyte differentiation, was assessed by ELISA. There was no significant difference in the amount of BAT between the three groups ($p=0.3$) and no association between BAT and BMI ($p=0.7$). A trend of an inverse association between BAT and age ($r=-0.50$, $p=0.06$) was seen. There was a positive correlation between BAT and BMD of the femoral neck ($r=0.63$, $p=0.01$), total hip ($r=0.58$, $p=0.02$), lumbar spine (LS) ($r=0.70$, $p=0.004$), and total body ($r=0.59$, $p=0.02$), which remained significant after controlling for age and disease status. BAT was inversely associated with log VAT ($r=-0.56$, $p=0.03$), while there was no association with other fat depots or lean mass. Subjects were divided into groups based on the presence ($n=7$) or absence ($n=8$) of BAT. Both groups were of comparable age and BMI. Women with BAT had higher lateral LS BMD (0.8 ± 0.08 vs $0.7 \pm 0.09 \text{ g/cm}^2$, $p=0.02$) and higher total body BMD (1.1 ± 0.05 vs $1.0 \pm 0.07 \text{ g/cm}^2$, $p=0.01$) compared to women without BAT. Women with BAT had lower Pref-1 compared to women without BAT (0.31 ± 0.15 vs $0.58 \pm 0.26 \text{ ng/ml}$, $p=0.03$). In conclusion, BAT is a positive predictor of BMD and is inversely associated with VAT, in young non-obese women.

Sources of Research Support: NIH grants R24 DK084970 and UL1 RR025758.

Nothing to Disclose: MAB, PKF, LMF, GC, WC, AHS, CJR, AK

Pub # P3-111

Session Information POSTER SESSION: CLINICAL - Osteoporosis, Fractures & Bone Density (1:30 PM-3:30 PM)

Title High-Resolution MRI, Vertebral Fractures and Osteoporosis in Men with Prostate Cancer

Author String N Gupta, JM Wagner, N Resnick, S Perera, ME Miller, JB Nelson, SL Greenspan
University of Pittsburgh, Pittsburgh, PA; University of Pittsburgh, Pittsburgh, PA; University of Pittsburgh, Pittsburgh, PA; University of Pittsburgh, Pittsburgh, PA

Body Androgen deprivation therapy (ADT), a common treatment for prostate cancer, is associated with increased bone loss and fracture. Although bone mineral density (BMD) assessed by conventional dual energy x-ray absorptiometry (DXA) is the gold standard to determine fracture risk, BMD only explains 60% of fracture risk. DXA is not routinely used to identify new vertebral fractures (VF). High resolution microMRI (HR-MRI) is a new technique to assess bone microarchitecture and may provide additional information on fracture risk. We postulated that 1) assessment for VF would increase the diagnosis of osteoporosis in men with prostate cancer on ADT and 2) assessment of trabecular microstructure by HR-MRI could demonstrate skeletal deterioration in men with VF, typically not detected by conventional BMD.

Methods: 100 men [ge] 65 years with nonmetastatic prostate cancer on ADT for [ge]6 months were enrolled in a case-control study. BMD of the spine and hip were assessed by DXA and classified using standard WHO criteria for osteoporosis. Each patient underwent vertebral fracture assessment (VFA) by DXA and confirmed by conventional lateral x-ray. HR-MRI of the wrist was also performed.

Results: VF were found in 34%. Duration of ADT correlated (r) with lower BMD at the spine (r = - 0.26), hip (r = - 0.22) and wrist (r = - 0.23, all p<0.05). 33% of men without osteoporosis by BMD had VF. 77% of patients with clinical osteoporosis would have been misclassified by BMD alone. BMD at the femoral neck was lower in men with VF compared to those without (p<0.05). Indices of HR-MRI including bone to total volume ratio, surface density (represents trabecular plates) and surface/curve ratio (plates/rods) were lower in patient with VF (p<0.1). Indices of HR-MRI were associated with BMD of the spine (r = 0.27 to 0.37); hip (r = 0.44 to 0.50) and wrist (r = 0.38 to 0.66, all p <0.05).

Conclusion: BMD misclassifies the majority of older men with prostate cancer on ADT but adding VF assessment to DXA analyses can substantially reduce such misclassification. HR-MRI provides a novel technique to assess skeletal integrity and adds additional information to conventional DXA.

Sources of Research Support: 2K24DK062895-06, PC060710 (DOD IDEA), University of Pittsburgh Clinica Translational Research Center RFA-RM-06-002, Claude D. Pepper Center, Division of Geriatric Medicine 2 P30 AG024827-06, University of Pittsburgh Dept of Urology.

Nothing to Disclose: NG, JMW, NR, SP, MEM, JBN, SLG

Pub # P3-112

Session Information POSTER SESSION: CLINICAL - Osteoporosis, Fractures & Bone Density (1:30 PM-3:30 PM)

Title Atypical Femoral Fractures during Prolonged Use of Bisphosphonates: Short-Term Responses to Strontium Ranelate and Teriparatide

Author String N Carvalho, D Fontan, L Voss, C Lucena, F Bandeira
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Body Introduction: Atypical femoral fractures have rarely been reported in women taking bisphosphonate. The aim of this study is to report 3 such cases and their responses to the use of strontium ranelate (SR) and teriparatide
Case 1: A 63-year-old woman presented a unhealed subtrochanteric fracture of the left femur for 1 year. Alendronate had been taken for approximately 5 years and risedronate 35mg/w during the last year. Her serum 25-hydroxy-vitamin-D (s25(OH)D) was 16.2ng/ml. Zolendronate 5mg/yr and cholecalciferol 600U/d were started. At the end of the second year a radiographic callus was seen. T score femoral neck bone mineral density (FNBMD) was -1.6 and lumbar spine (LSBMD) was -2.5. After the third dose of zolendronate she had another fracture in the middle 1/3 of the right femur. Serum carboxyterminal telopeptide CTX (sCTX) was 190pg/ml, serum osteocalcin (sOC) 8.2ng/ml and SR 2g/d was started. After 2 months of SR, OC and CTX were increased by 125% and 100%, respectively, with total closure of the fracture. Case 2: A 79-year-old woman had been on alendronate 70mg/w for 10 years followed by 1 year off when she was placed on ibandronate 150mg/mo. After 3 years of its use she had a subtrochanteric fracture in the left femur which was not healed 10 months later. sCTX was 337pg/ml, sOC 12ng/ml, s25(OH)D 14.7ng/ml. Cholecalciferol 20000U/w and SR 2g/d were started. After 3 months healing of the fracture line was achieved, sCTX and sOC increased 22% and 50%, respectively. Case 3: A 77-year-old woman presented with a 1 year unhealed subtrochanteric fracture of the right femur. She was on risedronate 35mg/w, calcium carbonate 1g/d and cholecalciferol 800U/d for 2 years. She presented with FNBMD -2.5, sCTX 211pg/ml, sOC 14.6ng/ml, s25(OH)D 37.4ng/ml. After 1 month on Teriparatide 20[micro]g/d a radiographic closure of fracture was achieved and after 3 months sCTX and sOC increased by 22% and 300%, respectively. Conclusion: Our data showed a rapid bone anabolic effect of both teriparatide and SR on unhealed atypical fracture associated with chronic bisphosphonate use.

Nothing to Disclose: NC, DF, LV, CL, FB

Pub # P3-113

Session Information POSTER SESSION: CLINICAL - Osteoporosis, Fractures & Bone Density (1:30 PM-3:30 PM)

Title Risk of New Vertebral Fractures in Patients with Adrenal Incidentaloma with and without Subclinical Hypercortisolism: A Multicenter Longitudinal Study

Author String V Morelli, C Eller Vainicher, AS Salcuni, F Coletti, L Iorio, G Muscogiuri, S Della Casa, M Arosio, B Ambrosi, P Beck-Peccoz, I Chiodini
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Body **Background.** In patients with adrenal incidentalomas (AI), cross-sectional case-control studies suggested that subclinical hypercortisolism (SH) is associated with an increased prevalence of vertebral fractures and spinal deformity index (SDI), a clinical index of reduced bone quality. However, to date no longitudinal studies are available investigating the incidence of vertebral fractures and SDI changes over time in SH patients. **Aim.** To evaluate in AI patients vertebral fractures' risk and SDI changes during 24 months follow-up period. **Methods.** In 103 consecutive AI patients (35 M, 68 F) at baseline, after 12 and 24 months pituitary-adrenal axis secretion and bone status were evaluated. SH was diagnosed in the presence of [ge]2 among: urinary free cortisol (UFC) >70 [mu]g/24h, serum cortisol after 1-mg dexamethasone suppression test (1mg-DST) >3.0 [mu]g/dL, ACTH <10 pg/mL, in [ge]2 out of the 3 evaluations. Patients were divided in SH- (n=76) and SH+ (n=27) groups. At baseline and after 24 months, bone mineral density (BMD) by Dual-energy X-ray Absorptiometry, the presence of vertebral fractures by the semi-quantitative method and the spinal deformity index (SDI, a surrogate clinical marker of bone quality obtained by summing the grade of deformity for each vertebra) were evaluated. **Results.** At the end of follow-up, SH+ group showed a higher prevalence of vertebral fractures (81.5%) as compared to baseline (55.6%, $P=0.04$), and a worsening of SDI (2.11 ± 1.85 vs 1.11 ± 1.47 , $P=0.032$), the latter being associated with SH regardless of age, gender, body mass index, BMD, SDI at baseline, years since menopause (OR 12.3, 95%CI 4.1-36.5, $P=0.001$). The incidence of new vertebral fractures was higher in SH+ than in SH- group (48% vs 13% respectively, $P=0.001$). **Conclusion.** in AI patients, SH is associated with the risk of developing new vertebral fractures and with a deterioration of bone quality.

Nothing to Disclose: VM, CEV, ASS, FC, LI, GM, SDC, MA, BA, PB-P, IC

Pub # P3-114

Session Information POSTER SESSION: CLINICAL - Osteoporosis, Fractures & Bone Density (1:30 PM-3:30 PM)

Title High Prevalence of Radiological Vertebral Fractures in Patients with Heart Failure

Author String G Mazziotti, M Baracca, PP Vescovi, A Giustina
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Body Heart failure (HF) has been associated with bone loss and an increased risk of clinical fractures. Indeed, data on fractures were focused on retrospective historical assessments of symptomatic fractures registered in clinical databases. Vertebral fractures are the most common osteoporotic fractures and are largely underdiagnosed based upon clinical records, since most of them are asymptomatic. In this study we investigated the prevalence of vertebral fractures in patients with HF, using a radiological and morphometric approach performed on chest radiography. Exclusion criteria were: 1) autoimmune diseases; 2) neoplastic diseases; 3) chronic treatment with oral glucocorticoids; 4) chronic immobilization. The study involved 791 consecutive subjects (372 females, 419 males, median age 75 years, range: 22-95) referring to the Department of Medicine of our Institutions. All patients underwent a standard posteroanterior and lateral chest radiography. HF was diagnosed in 190 patients (24.0%), who were significantly older than patients without HF (82 yrs, range: 48-95 vs. 71 yrs, range: 22-88; $p<0.001$). Vertebral fractures were observed in 170 patients (21.5%). Seventy-one patients had two or more fractures and 42 patients had severe fractures, as defined according to Genant score. The prevalence of vertebral fractures was significantly higher in patients with HF as compared to those without HF (34.7% vs. 17.3%; $p<0.001$). Moreover, patients with HF showed more frequently multiple fractures than patients without HF. Among patients with HF, those with vertebral fracture had higher number of disease recurrences over the last 5 years as compared to patients who did not fracture (3 range 1-8 vs 1, range: 1-6; $p<0.001$). The multivariate analysis in the whole population demonstrated that vertebral fractures were significantly associated with HF (odds ratio: 2.4, C.I.95% 1.4-3.9; $p=0.01$) independently of age, sex, diuretic therapy, anticoagulant therapy, proton pump therapy, coexistent chronic obstructive pulmonary disease, diabetes mellitus and chronic liver diseases. In conclusion, this study demonstrates for the first time that chronic HF may be frequently complicated by thoracic vertebral fractures. Therefore, long-term osteo-metabolic follow-up of these high risk patients is recommended.

Nothing to Disclose: GM, MB, PPV, AG

Pub # P3-115

Session Information POSTER SESSION: CLINICAL - Osteoporosis, Fractures & Bone Density (1:30 PM-3:30 PM)

Title Chronic Dioxin Intake and Osteoporosis in a Community-Based Population Residing near a Deserted Polychlorophenol Factory

Author String S-L Wang, C-C Lee, P-C Liao, S-Y Lee, J-W Chen, S-F Tsai, S-H Liou
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Body
Introduction: Osteoporosis is the most common metabolic bone disorder causing fragility fractures and is increasingly prevalent and costly worldwide. Dioxins are ubiquitous, have a half life of 7-10 years in human body, and exhibit adverse effects on bone mineralization, size, and strength in treated animals. The purpose is to examine the association between osteoporosis and chronic dioxin intake in humans.
Materials and Methods: A total of 3316 residents were interviewed in their homes between March 2008 and June 2010 in a contaminated area where a pentachlorophenol factory operated from 1967 to 1982. Osteoporosis was defined as having the notification from a clinician. A total 1768 subjects over 20 years of age have 40 ml blood drawn for the analyses of the WHO 17 polychlorinated dibenzo-dioxins and dibenzofurans using high-resolution gas-chromatography/mass spectrometry.
Results: A significant odds ratio of 4.3 was found for those with levels of 33-63 pg WHO(1998)-TEQ/g lipid as compared to [le]32 pg in men aged [le]50 years born in 1961-1990. In those aged 50-59 years, developing puberty in 1962-1972, significant odds ratios of 5.2 for men and 11.7 for women with dioxin levels [ge]64 pg compared to [le]32 pg after consideration of cigarette smoking, exercise, nutrient supplement, and menopause
Conclusions: This is the first report on the association between of increased risk of osteoporosis and dioxin in humans exposed at early days of life or before puberty. Avoiding the exposure of such persistent organic pollutants with anti-estrogenic attribute is important for prevention of osteoporosis in later life.

Sources of Research Support: Tainan Department of Health (DOH 97D9-EOTN01) and the National Science Council (NSC 97-2314-B-400-007-MY3).

Nothing to Disclose: S-LW, C-CL, P-CL, S-YL, J-WC, S-FT, S-HL

Pub # P3-116

Session Information POSTER SESSION: CLINICAL - Osteoporosis, Fractures & Bone Density (1:30 PM-3:30 PM)

Title Preadipocyte Factor-1 Is Associated with Bone Mineral Content and Bone Mineral Density in Women with Exercise-Induced Hypothalamic Amenorrhea Independently of Leptin Levels

Author String KN Aronis, H Kilim, JP Chamberland, A Breggia, C Rosen, CS Mantzoros
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Body
Introduction: Preadipocyte factor-1 (pref-1) is increased in anorexia nervosa and is negatively associated with bone mineral density (BMD). No prior studies exist on pref-1 in women with exercise-induced hypothalamic amenorrhea (HA), another hypoleptinemic state. Our objective was to evaluate whether pref-1 levels are also associated with low BMD in women with HA, and to assess whether leptin regulates pref-1 in humans.
Methods: We conducted 2 separate studies; (1) Study 1 was a double-blinded, placebo-controlled, randomized clinical trial of metreleptin administration, where 20 females with HA and hypoleptinemia (leptin levels < 5ng/ml) were randomized to received 0.08 mg/kg of either metreleptin qd (n=11) or placebo (n=8) for 36 weeks. (2) Study 2 was an open-label study, where 5 healthy females received 0.01 mg/kg, 0.1 mg/kg, and 0.3mg/kg of metreleptin in both fed and fasting conditions, for one and three days respectively. Main outcome measures included circulating pref-1 and leptin levels, as well as BMD and bone mineral content (BMC). Statistical analysis: Variables were examined for normality and skewed variables were normalized with the appropriate transformation. Non-parametric statistics were used where normalization failed. Associations between variables of interest were examined with spearman's Correlations Comparison between the placebo and leptin group of study 1, over time was conducted with Hierarchical Linear Modeling Analysis. Study 2 samples were analyzed with Friedman's two way Analysis of Variance and Wilcoxon signed rank test where appropriate.
Results: Pref-1 was found to be significantly higher in HA subjects vs. controls ($p = 0.035$) and negatively associated with BMD ($[\rho] = -0.38, p < 0.01$) and BMC ($[\rho] = -0.32, p < 0.05$). Metreleptin administration did not alter pref-1 levels at any dose or duration utilized in this study.
Conclusions: Pref-1 is higher in HA subjects than controls. Leptin administration at low, replacement, physiologic and pharmacologic doses does not affect pref-1 levels, suggesting that hypoleptinemia is not responsible for lower pref-1 levels and that leptin does not regulate pref-1.

Sources of Research Support: National Institutes of Health - National Center for Research Resources grant M01-RR-01032 (Harvard Clinical and Translational Science Center) and grant number UL1 RR025758. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Center for Research Resources or the National Institutes of Health. Funding was also received from the National Institute of Diabetes and Digestive and Kidney Diseases grants 58785, 79929 and 81913, and AG032030. Amylin Pharmaceuticals, Inc. supplied metreleptin for this study but had no role in the study design; conduct of the study; collection, management, analysis, and interpretation of the data; or the preparation, review, or approval of the manuscript.

Nothing to Disclose: KNA, HK, JPC, AB, CR, CSM

Pub # P3-118

Session Information POSTER SESSION: CLINICAL - Osteoporosis, Fractures & Bone Density (1:30 PM-3:30 PM)

Title Prevalence of Free Light Chain Abnormalities in Patients Undergoing Evaluation for Osteoporosis

Author String A Varughese, G Jaiswal, I Khanna, EM Uy, L Cirullo, F Mirza
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Body

Background: Multiple secondary factors may exacerbate bone loss after menopause, including monoclonal gammopathy of uncertain significance (MGUS), which routine lab testing may not always detect. Serum free light chain (FLC) assay is a relatively new and sensitive test that evaluates abnormalities in FLC. An immunoglobulin molecule has either kappa (KLC) or lambda (LLC) light chains. Forty percent more light chains are produced compared to the heavy chains. Excess FLC are released into the serum and cleared by the kidneys. We assessed FLC in patients with recurrent fractures or progressive bone loss despite treatment and noted a high frequency of abnormal results. To our knowledge, there are no reported studies that have evaluated the prevalence of such abnormalities in osteoporosis.

Objective: Evaluate the prevalence of free light chain abnormalities in patients with osteoporosis.

Design: Retrospective study of patients with osteoporosis who had FLC test done between January 2009 and June 2010.

Results: Data on 72 patients were reviewed. Mean age of subjects was 70 ± 12 years, BMI was 24 ± 6 kg/m², and creatinine clearance (CrCl) was 60 ± 26 ml/min. 25 hydroxy vitamin D levels were 42 ± 13 ng/ml and urine NTX was 30 ± 16 units. 83% patients were on osteoporosis treatment with bisphosphonates (BP), 60 % had prior history of fractures and 12 % had a prior history of malignancy. Age was a negative predictor of CrCl and bone mineral density (BMD) at the total hip (TH, $r = -0.556$, $p < 0.001$) and femoral neck (FN, $r = -0.479$, $p < 0.001$). CrCl correlated positively with BMD at TH and FN ($r = 0.611$, $p < 0.001$, $r = 0.557$, $p < 0.001$ respectively), indicating that bone density was higher with higher CrCl. Of the 72 subjects, 46% had an abnormal K:L ratio above the upper limit of normal, with 3 subjects having a ratio of >4 , who were later confirmed to have non secretory myeloma. Although LLC correlated negatively with the CrCl ($r = -0.386$, $p = 0.002$) we did not find any relationship b/w the KLC and K:L ratio to kidney function. The KLC had a significant negative correlation with the FN ($r = -0.306$, $p = 0.02$) but not the TH BMD.

Conclusions: There is a high incidence of abnormalities of the free light chain ratio in patients with severe osteoporosis and in poor responders to osteoporosis treatment, suggesting that an abnormal K:L ratio may be an independent risk factor for severe osteoporosis. These findings need to be confirmed in larger prospective studies.

Sources of Research Support: Joanne Smith MD Memorial Research and Education Fund; University of Connecticut.

Nothing to Disclose: AV, GJ, IK, EMU, LC, FM

Pub # P3-119

Session Information POSTER SESSION: CLINICAL - Osteoporosis, Fractures & Bone Density (1:30 PM-3:30 PM)

Title Bone Density and FGF23 in Leptin Deficient Patients with Congenital Generalized Lipodystrophy: Comparison to the Animal Models

Author String AO Lungu, WH Chong, E Safar Zadeh, EK Cochran, J Reynolds, MT Collins, P Gorden
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Body Background: The adipocyte-derived hormone leptin has been shown to be important in bone density and the regulation of the phosphate and vitamin D- regulating hormone, FGF23, in various in vivo and in vitro models. Leptin-deficient and leptin receptor-deficient mice have increased bone density that is reversed by leptin therapy, and leptin directly increased FGF23 expression in bone cells and in animal models. Patients with congenital generalized lipodystrophy (CGL) have very low leptin levels, severe insulin resistance, and hypertriglyceridemia. To investigate the role of leptin in human bone and mineral physiology, we analyzed bone density and FGF23 levels in the NIH cohort of subjects with CGL before and after leptin therapy. Subjects and Methods: Twenty-two subjects with CGL (baseline age 8-41, mean 16) were treated with leptin at a dose necessary to normalize glucose and triglyceride metabolism (0.06 - 0.24 mg/kg/d, sq) for at least one year. Whole body bone density was measured by dual x-ray absorptiometry (Hologic, Waltham, MA) and normalized by using Z-Scores, and intact FGF23 was measured by ELISA (Kainos, Tokyo, Japan). Baseline, 1 and 3-5 years values were compared. Results: Bone density at baseline was elevated; Z-Score = 3.58 ± 1.32 . However, leptin treatment, regardless of length of treatment, had no effect on bone mass (Z-Score = 3.63 ± 1.02). Mean FGF23 levels at baseline were normal, 28 ± 13 pg/ml and unchanged after 1 year of treatment, 30 ± 13.5 pg/ml. There were no significant differences in vitamin D, osteocalcin, alkaline phosphatase and calcium levels at baseline or after leptin therapy. Leptin therapy was effective in lowering triglycerides and HbA1C in all patients. Of note, 6/18 female patients were hyperandrogenemic at baseline and the testosterone level significantly decreased after 1 year of leptin therapy. Conclusion: Similar to what is seen in leptin-deficient rodents, leptin deficiency in subjects with CGL was associated with high bone mass. However, confounding the interpretation of the effect of leptin on bone density is the fact that some subjects were hyperandrogenemic at baseline. Inconsistent with a significant effect of leptin on bone mass is the fact that leptin replacement had no effect on bone mass. Neither leptin deficiency nor treatment had any effect on plasma FGF23 levels. These data leave open the question of the effect of leptin on bone density, and suggest leptin has no effect on FGF23 regulation in humans.

Nothing to Disclose: AOL, WHC, ESZ, EKC, JR, MTC, PG

Pub # P3-120

Session Information POSTER SESSION: CLINICAL - Osteoporosis, Fractures & Bone Density (1:30 PM-3:30 PM)

Title Comparison of High-Resolution Peripheral Quantitative Computerized Tomography (HR-pQCT) with Dual Energy Photon Absorptiometry (DXA) for Measuring Bone Mineral Density (BMD) in Subjects of Different Fatness

Author String E Colt, M Akram, J Thornton, D Beiling, M Jerome, A Kausel, S Paladugu, R Tajrishi, Z Servetnyk, FX Pi-Sunyer
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Body We were interested to compare the measurement of BMD by HR-pQCT with that measured by DXA in subjects varying from very thin (BMI 20) to morbidly obese (BMI > 40). During the last two decades, DXA has been the mainstay for measuring BMD in people of all builds. However it has been noted that BMD measured by DXA becomes progressively more inaccurate with increasing obesity. There is evidence that obesity reduces (real) BMD compared with subjects of same weight who are not obese. It is thus important to improve the measurement of BMD of patients who lose weight because low BMD predicts future osteoporotic fracture. HR-pQCT provides information about the structure of bone: These structural parameters (measured by HR-pQCT) may be relatively unaffected by fat. DXA does not give any information about bone structure, and only provides BMD, and total bone mineral content. We wished to see if HR-pQCT may provide more information and be less artifactually affected than DXA for measuring BMD, in obese subjects. We wish to report our preliminary results on a cross sectional study of 27 healthy pre-menopausal women aged 18 - 45, ranging in BMI from 20 to 46.

METHODS: BMD and surrogates thereof measured by HR-pQCT (XtremeCT Scanco Medical) and those measured by DXA (GE Lunar iDXA in total body mode) were plotted against BMI to compare the correlation coefficients (CC) with the two methods.

RESULTS. The CC of BMI vs radius average BMD by qCT was 0.29. The CC of BMI vs radius cortical thickness (a BMD surrogate by qCT) was 0.30. The CC of BMI vs arm BMD by DXA was 0.87. The CC of BMI vs tibia average BMD by qCT was 0.08; the CC of BMI vs tibia cortical thickness (a BMD surrogate by qCT) was 0.32. The CC of BMI vs leg BMD by DXA was 0.64. Average BMD by qCT includes trabecular BMD, compact bone density, cortical thickness, and other parameters, all of which had lower CCs with BMI than did measurements of arms and legs by DXA.

INTERPRETATION The difference in CCs by the two methods can be explained by a difference in the effect of fat in causing an artifactual increase in BMD. IE this artifact is greater in DXA than in qCT.

CONCLUSION This study suggests but does not prove that HR-qCT is more accurate for comparing BMD in people of differing adiposity.

CONCLUSION This study suggests but does not prove that HR-pQCT is more accurate than DXA for comparing BMDs in people of differing adiposity.

Sources of Research Support: NIH grant DK026687-27.

Nothing to Disclose: EC, MA, JT, DB, MJ, AK, SP, RT, ZS, FXP-S

Pub # P3-121

Session Information POSTER SESSION: CLINICAL - Osteoporosis, Fractures & Bone Density (1:30 PM-3:30 PM)

Title The Effects of Rosiglitazone and Metformin on Longitudinal Changes in Bone Mineral Density and Bone Turnover Markers in Non-Diabetic HIV-Infected Persons with Lipodystrophy: A Secondary Analysis of Aids Clinical Trial Group (ACTG) A5082

Author String V Walker Harris, G McComsey, H Chen, L Deng, M Yin, S Cremers, S Grinspoon, K Mulligan, T Brown Johns Hopkins University School of Medicine, Baltimore, MD; Case School of Medicine, Cleveland, OH; Harvard School of Public Health, Boston, MA; Columbia University School of Medicine, New York, NY; Massachusetts General Hospital, Boston, MA; University of California, San Francisco, San Francisco, CA

Body

Background
Osteoporosis is common among HIV+ persons with a multifactorial pathogenesis. Rosiglitazone, a medication investigated for the treatment of lipodystrophy, is associated with lower bone mineral density (BMD) and fracture among HIV-negative persons with diabetes. Metformin, also investigated for the treatment of fat abnormalities in HIV, was associated with decreased levels of bone formation markers in the ADOPT study (1). We conducted a secondary analysis of a completed randomized, clinical trial (2) to compare the effects of rosiglitazone, metformin, or placebo on BMD and bone turnover in HIV-infected persons with lipodystrophy and insulin resistance.

Methods
Of the 105 HIV-infected subjects initially enrolled, 70 had samples available at baseline and 16 weeks for measurement of procollagen type 1 amino-terminal propeptide (P1NP), a bone formation marker, or c-telopeptide (CTX), a bone resorption marker. These included 21 in the Rosiglitazone/Placebo group (R/P), 12 in the Metformin/Placebo group (M/P), 19 in the Rosiglitazone/Metformin group (R/M), and 18 in the dual placebo group (P/P). The change in whole body BMD and bone turnover markers over 16 weeks was compared in the 4 groups. Multivariable linear regression was used to determine: 1) the independent effects of rosiglitazone and metformin on the change in P1NP and CTX, and 2) whether the change in P1NP or CTX was associated with the change in BMD.

Results
The percentage change in whole body BMD over 16 weeks was similar between the four groups (median change: R/P -0.50%, M/P 0.29%, R/M 0.33%, P/P -0.16%), without an independent effect of rosiglitazone or metformin. There were no significant changes in CTX in any of the groups. P1NP however decreased significantly in the M/P (-20.23%, p=0.02) and R/M (-23.92%, p=0.002) groups over 16 weeks. In multivariable analyses, metformin (p=0.03), but not rosiglitazone (p=0.55) was associated with a decrease in P1NP. The change in P1NP was not correlated with the change in lower extremity fat or insulin resistance. Among those on metformin, the change in P1NP was not associated with changes in weight or BMD.

Conclusion
In HIV+ persons with lipodystrophy, rosiglitazone was not associated with changes in whole body BMD, bone resorption, or bone formation. The mechanisms and clinical significance of the observed effect of metformin decreasing the bone formation marker, P1NP, deserve further investigation.

(1) Zinman et al., J Clin Endocrinol Metab 2010; 95:134
(2) Mulligan et al., AIDS 2007; 21:47

Nothing to Disclose: VWH, GM, HC, LD, MY, SC, SG, KM, TB

Pub # P3-122

Session Information POSTER SESSION: CLINICAL - Osteoporosis, Fractures & Bone Density (1:30 PM-3:30 PM)

Title Proton Pump Inhibitors and Risk of Fractures: A Meta-Analysis of 11 International Studies

Author String EW Yu, SR Bauer, PA Bain, DC Bauer
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Body Background: Concerns have been raised about the risk of fractures with acid-suppressive medications, such as proton pump inhibitors (PPIs) and histamine2-receptor antagonists (H2RAs).
Methods: In this meta-analysis, we evaluated the association between PPI or H2RA use and fractures. We performed a systematic search of published literature (1970 to October 10, 2010) in MEDLINE, EMBASE, and other sources. Ten publications reporting 11 studies were considered eligible for analysis.
Results: All studies were observational case-control or cohort studies and primarily evaluated older adults. The summary effect estimate for risk of hip fracture was modestly increased among individuals taking PPIs (RR 1.30, 95% CI 1.19-1.43). There was also an increase in spine (RR 1.56, 95% CI 1.31-1.85) and any-site fractures (RR 1.16, 95% CI 1.04-1.30) among PPI users. These findings were similar in both men and women and after stratification by duration of use. In contrast, H2RA use was not significantly associated with increased risk of hip fracture (RR 1.10, 95% CI 0.94-1.30).
Conclusions: In this meta-analysis of observational studies, PPIs modestly increased the risk of hip, spine, and any-site fractures, whereas H2RAs were not associated with fracture risk. The possibility of residual confounding cannot be excluded. Further skeletal evaluation should be considered for patients who are taking PPIs and are also at risk for osteoporotic fracture.

Sources of Research Support: NIH grant K24 ARO51895 awarded to DCB.

Nothing to Disclose: EWY, SRB, PAB, DCB

Pub # P3-123

Session Information POSTER SESSION: CLINICAL - Osteoporosis, Fractures & Bone Density (1:30 PM-3:30 PM)

Title Neuroendocrine Tumor Associated with Severe Osteoporosis in a Male Patient

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Body

Introduction
Gut-derived serotonin has recently emerged as an important regulator of bone mass. Serotonin inhibits bone formation by acting directly on osteoblasts. Neuroendocrine tumors may be able to produce serotonin. We report a case of severe osteoporosis as a manifestation of a neuroendocrine tumor.

Case Report
A 55-year-old male was referred to the endocrine clinic for evaluation of osteoporosis. About eight years prior, he had suffered a hip fracture after falling from his own height. Four months later, he fell again and suffered hip fracture on the other side. Two years after the first fracture, he experienced severe back pain and a vertebral fracture was diagnosed. One year later, he had two rib fractures following minor trauma. Dual energy x-ray absorptiometry revealed marked osteoporosis (T score: L1-L4 -4.5; neck -2.8; total femur -3.2). The patient had a history of nephrolithiasis and repeated episodic diarrhea. The investigation of malabsorption detected no abnormalities. Because of an initial measurement of phosphorus in the lower range (2.6 mg/dL; NR:3.0g/dL), an Octreoscan was performed and revealed a neuroendocrine tumor on the epigastrium, probably on the pancreas. Multi-slice CT was normal, but endoscopic ultrasonography found a lesion on the head of the pancreas. The patient underwent exploratory surgery, but the tumor was not found. The biochemical evaluation revealed normal calcium, PTH, alkaline phosphatase, 25OH vitamin D and creatinine. Hypogonadism and Cushing's disease were also ruled out. Phosphorus was repeated a number of times and was always normal. In the 24-hour urine, 5-hydroxyindoleacetic acid was normal and serum chromogranin A was doubtful (9.6 nmol/L; normal < 4.0; doubtful 4 - 10; elevated > 10nmol/L). An ELISA kit (Fitzgerald) revealed an increase in circulating levels of serotonin (90.3 ng/mL) in comparison to control subjects (mean: 29.5 ng/mL). Bone biopsy revealed significant low-turnover osteoporosis with reduced trabecular bone volume, low numbers of osteoclasts and osteoblasts and a reduced amount of osteoid.

Discussion
We described a case of severe osteoporosis in a male patient who was found with a serotonin-producing neuroendocrine pancreatic tumor. The late onset of multiple fractures in a previously healthy man, whose biopsy showed a low turnover osteoporosis agrees with the new paradigm of bone metabolism, in which gut-derived serotonin would be an important inhibitor of osteoblast function.

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Nothing to Disclose: TV, RMY, ABC, ML-C

Pub # P3-124

Session Information POSTER SESSION: CLINICAL - Osteoporosis, Fractures & Bone Density (1:30 PM-3:30 PM)

Title Bone Mineral Density Is Lower in Patients with Congenital Adrenal Hyperplasia Than in Patients with Acquired Primary Adrenal Insufficiency

Author String M Quinkler, M Ventz, S Diederich, KR Koetz
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Body Introduction: Patients with primary adrenal insufficiency (Addison[acute]s disease) and patients with congenital adrenal hyperplasia (CAH) still tend to receive more glucocorticoids than the normal endogenous production in healthy subjects. CAH patients start treatment usually with diagnosis in their early childhood, whereas Addison[acute]s patients have a later onset of their disease and start of treatment. Objective: To compare patients with Addison[acute]s disease and CAH in regard to bone mineral density (BMD), duration of glucocorticoid therapy and impact of glucocorticoid pharmacogenetics. Methods: In a cross-sectional study patients from an university endocrine outpatient clinic were included (84 pat with Addison[acute]s disease, 42 pat with CAH). BMD was measured using DXA scan. Blood samples were analyzed for bone markers and 24h urinary samples were analyzed for bone resorption markers. Results: Patients with Addison[acute]s disease were significantly older ($55.1\pm 15.2y$ vs $39.9\pm 13.8y$, $p<0.001$) and taller ($168\pm 10cm$ vs $160\pm 11cm$, $p<0.001$) than CAH patients, but showed no difference in BMI. Time since diagnosis was shorter in Addison[acute]s patients ($15.1\pm 10.6y$ vs $30.5\pm 13.5y$, $p<0.001$). The calculated hydrocortisone equivalent glucocorticoid dose per body surface was higher in CAH than Addison[acute]s patients ($16.0\pm 7.4mg/m^2$ vs $12.0\pm 2.7mg/m^2$, $p<0.001$). No differences were seen in serum Ca, aP, ostase, PTH, 25-vitaminD3, 1,25-vitaminD3, osteocalcin or urinary cross-links. Femoral neck BMD Z-scores were significantly lower in patients with CAH compared to Addison[acute]s disease (-0.59 ± 1.17 vs 0.01 ± 0.97 , $p<0.01$); also Z-scores for femoral Ward[acute]s region were lower in CAH (-0.96 ± 1.04 vs -0.28 ± 1.02 , $p<0.005$). No difference was found in lumbar spine Z-scores. Conclusions: BMD at femoral neck and femoral Ward[acute]s region were lower in CAH than Addison's disease patients, indicating undesirable effects of higher glucocorticoid dose, usage of longer acting glucocorticoids, and longer duration of replacement therapy.

Nothing to Disclose: MQ, MV, SD, KRK

Pub # P3-125

Session Information POSTER SESSION: CLINICAL - Osteoporosis, Fractures & Bone Density (1:30 PM-3:30 PM)

Title Bone Mineral Density Testing in Male Patients: Reasons for Referral and Effect on Clinical Management

Author String VM Bota, M Menon, SE Reinert, G Gopalakrishnan
Boston University School of Medicine, Providence, RI; Alpert Medical School of Brown University, Providence, RI; Rhode Island Hospital, Providence, RI

Body **Background:** Despite the significant mortality and morbidity associated with osteoporotic fractures, osteoporosis remains under-diagnosed and under-treated in men.
Methods: Between 2002 and 2009 we conducted a retrospective chart review of male patients referred for Bone Mineral Density (BMD) testing using dual energy X-ray absorptiometry (DEXA scan). We collected age, race, BMI, referring physician, reason for referral, medical history, medications and bone density results, and evaluated reasons for the initial referral and factors that influenced repeat testing and treatment initiation.
Results: 350 patients (mean age of 54.9 years) were included in the study. Most patients were referred by the PCP (51.3%). Significant risk factors included Caucasian race (81.1%), age >70 (20.1%), history of fractures (34.9%), steroid use (26.9%), anticonvulsants use (14.6%), hypogonadism (14.0%), malabsorption (10.6%), hyperparathyroidism (7.4%), low BMI (4.5%) and aromatase-inhibitor use (3.4%). 84.57% of the study population reported a secondary cause rather than age related screening. Less than half of the patients were already on vitamin D, and only one-third reported calcium supplementation at baseline. 26.0% of the subjects met criteria for osteoporosis, 49.7% had osteopenia, and 24.3% had a normal test. Only 113 patients (32.3%) returned for repeat testing. 89.4% of these patients had an abnormal test result at baseline. In this group, a significant increase in osteoporosis-related treatment (vitamin D, calcium, bisphosphonates) was noted, compared with the previous visit (68.1% vs. 50.4%, 69.0% vs. 42.5% and 25.7% vs. 4.4% respectively - p<0.001). Patients referred by an Endocrinologist, or those treated with calcium and vitamin D supplementation were more likely to have a second BMD test on multivariate analysis.
Conclusion: Men were referred for DXA testing due to risk factors rather than routine age-related screening. Despite the low rate of follow-up testing, a significant increase in osteoporosis treatment was noted after the initial visit. Strategies to improve screening in our aging male population are needed.

Nothing to Disclose: VMB, MM, SER, GG

Pub # P3-126
Session Information POSTER SESSION: CLINICAL - Osteoporosis, Fractures & Bone Density (1:30 PM-3:30 PM)
Title Skin Wrinkling and Rigidity Are Predictive of Bone Mineral Density in Early Postmenopausal Women
Author String L Pal, N Kidwai, K Glockenberg, N Schneller, T Altun, A Figueroa, B Isaac, L McDonald, L Lipton, E Wolff H Taylor, NF Santoro, R Freeman
Yale University, New Haven, CT; University of Connecticut, Storrs, CT; Albert Einstein College of Medicine Bronx, NY; University of Colorado at Denver, Aurora, CO; NIH, Washington, DC

Body **Objective:** Relevance of collagen scaffold in maintaining tissue architecture is well described for the skin & the skeleton. Alterations in skin wrinkling & texture and reduced bone mineral density (BMD) are recognized accompaniments of reproductive & chronological aging. We explored the relationship between skin wrinkling or rigidity & BMD in a cohort of early menopausal women (within 3 years of last period) enrolled in a longitudinal trial of menopausal hormone therapy, the Kronos Early Estrogen Prevention Study (KEEPS), who additionally participated in a Skin Ancillary study.

Design: Cross-sectional analyses of baseline data from KEEPS.

Participants: 114 enrollees at two of the 9 participating study sites.

Interventions: Skin wrinkles were assessed at 11 sites on face & neck using the Lemperle wrinkle scale (1). Skin rigidity was assessed at the forehead & cheek using a durometer (2). Participants underwent BMD assessment by dual X-ray absorptiometry (DXA) at the lumbar spine, left hip & total body, & by quantitative heel ultrasound (QUS). Relationship of BMD to skin wrinkles & skin rigidity was assessed. Stepwise multivariable linear regression analyses explored the relationship between skin parameters & BMD; age, body mass, race & ethnicity, age at menopause, history of smoking, intake of multivitamins & enrollment site were used as covariates.

Results: Skin wrinkling showed an inverse relationship with BMD at the spine ($r = -0.27$, $p < 0.01$), femoral neck ($r = -0.29$, $p < 0.01$), total body BMD ($r = -0.26$, $p = 0.01$). In contrast, significant positive correlations were observed between skin rigidity, as reflected by the total durometer score (face + forehead), & BMD at the spine ($r = 0.40$, $p < 0.001$), left femoral neck ($r = 0.48$, $p < 0.001$) & with BUA (bone ultrasound attenuation by QUS) ($r = 0.28$, $p = 0.006$). Increasing forehead glabellar wrinkles were found to relate independently to lower femoral neck BMD ($AR^2 = 59$, $\beta = -0.019$, $SE = 0.009$, $p = 0.033$, $95\% \text{ CI} = -0.037 - 0.002$). Increasing skin rigidity at the face & forehead was identified as an independent determinant of BMD at the hip ($AR^2 = 42$, $\beta = 0.001$, $SE = 0.0002$, $p < 0.001$, $95\% \text{ CI} = 0.001 - 0.001$), at the spine ($AR^2 = 26$, $\beta = 0.001$, $SE = 0.0003$, $p < 0.001$, $95\% \text{ CI} = 0.0005 - 0.002$), & of BUA ($AR^2 = 0.10$, $\beta = 0.12$, $SE = 0.06$, $p = 0.039$, $95\% \text{ CI} = 0.006 - 0.232$).

Conclusion: In a population of early postmenopausal women, study of the skin is observed to provide a glimpse into the status of the skeleton, a relationship not previously described.

1. Lemperle G, Holmes RE, Cohen SR, Lemperle SM. A classification of facial wrinkles. *Plastic & Reconstructive Surgery*. 108(6):1735-50; discussion 1751-2, 2001 Nov

2. Wolff E, Narayan D, and Taylor H.S. *Fertil Steril*. 2005 Aug;84 (2):285-8.

3. Wolff E, Pal L, Altun T, Madankumar R, Freeman R, Amin H, Harman M, Santoro N, Taylor HS. Skin wrinkles and rigidity in early postmenopausal women vary by race/ethnicity: baseline characteristics of the skin ancillary study of the KEEPS trial.

Fertil Steril. 2010 Oct 23. [Epub ahead of print]

Sources of Research Support: Aurora Foundation to the Kronos Longevity Research Institute.

Nothing to Disclose: LP, NK, KG, NS, TA, AF, BI, LM, LL, EW, HT, NFS, RF

Pub # P3-127

Session Information POSTER SESSION: CLINICAL - Osteoporosis, Fractures & Bone Density (1:30 PM-3:30 PM)

Title Improving the Detection and Management of Fragility Fractures in Ambulatory Patients: The Fracture Capture Program

Author String CJ Yates, AT Bucknill, JD Wark
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Body Only 7-20% of patients in Australia presenting with osteoporotic fractures receive treatment despite the known morbidity, mortality and expense associated with these events (National Institute of Clinical Studies 2005). At our hospital, acute fracture patients not requiring hospitalisation are referred for outpatient care at orthopaedic fracture clinics. Before 2009, there was no osteoporosis policy at these clinics and audit revealed that bone mineral densitometry (BMD) occurred in 1 of 49 patients with fragility fractures. Patients aged over 65 years who were admitted to hospital with fractures received shared care with geriatricians, while those with a hip fracture were jointly managed with internal medicine physicians. Consequently the assessment and medical management of osteoporosis was significantly better for inpatients than outpatients with fragility fractures.

Aim: To improve the detection and management of osteoporosis in outpatients with fragility fractures.

Methods: In 2010, we designed and introduced a model to improve fragility fracture detection and medical management in outpatients. A part-time coordinator identified fracture clinic patients who were aged over 50 years and had sustained fragility fractures. These patients were given a letter explaining the need for an osteoporosis evaluation and investigated with BMD and a blood test (osteoporosis risk factors) before attending for physician osteoporosis assessment. If specific osteoporosis therapy was commenced then a 3 month review was made to assess administration technique and tolerability. Medication selection was tailored to patient preference, comorbidities and evidence base.

Results: 70 new patients with suspected fragility fractures were assessed between 7 April and 1 December 2010. The mean age was 67 years (SD=11) and 83% were female. The commonest fractures were at the wrist (30), humerus (12), foot (11) and ankle (7). Mean DXA T-scores were -1.4 at the lumbar spine, -1.4 at the total hip and -1.9 at the femoral neck. Mean 25 hydroxyvitamin D levels were 55nmol/L (SD=26). 40 patients were prescribed osteoporosis medication; 15 risedronate, 9 alendronate, 9 zoledronic acid, 7 strontium ranelate.

Conclusion: We have demonstrated an effective model to improve osteoporosis assessment and management in ambulatory orthopaedic patients. This model is reliant on external financial support at present and requires further funding support to be extended and generalised to other institutions.

Sources of Research Support: Servier Laboratories Australia.

Disclosures: CJY: Coinvestigator, Amgen; Clinician, Servier. ATB: Clinician, Servier. JDW: Clinician, Servier; Consultant, Amgen; Sanofi-Aventis; Novartis Pharmaceuticals; Servier; GlaxoSmithKline; Investigator, Eli Lilly & Company; Sanofi-Aventis; GlaxoSmithKline.

Pub # P3-128
Session Information POSTER SESSION: CLINICAL - Osteoporosis, Fractures & Bone Density (1:30 PM-3:30 PM)
Title Severely Decreased Bone Density in Graves Disease
Author String SE French, AW Gannon, J Subauste
University of Mississippi Medical Center, Jackson, MS; G V (Sonny) Montgomery Veterans Affairs Medical Center, Jackson, MS

Body Background: The majority of osteoporosis in men is associated with secondary causes such as hypogonadism, excessive alcohol intake and hyperthyroidism (1). Treatment of hyperthyroidism can improve bone mineral density in affected patients; however the bone disease may be complicated by other factors such as vitamin D deficiency.

Clinical case: A 48 year-old African American male was referred to endocrinology clinic for 18 months of uncontrolled hyperthyroidism with unintentional 20-kilogram weight loss, but otherwise healthy. TSH 0.01 uIU/mL (normal range 0.34-5.6), free T4 5.41 ng/dL (0.61-1.12), total T3 9.89 ng/mL (0.87-1.78), normal renal function, normal electrolytes with calcium 9.3 mg/dL (8.8-10.5) and phosphorus 4.1 ng/dL (1.8-2.4), alkaline phosphatase 383 U/L (38-126), and 25-hydroxy-vitamin D was 28.6 ng/mL (32-100). Radioactive iodine uptake and scan showed 75% uptake at 4 hours. Patient received radioactive iodine ablation with 20.8 milliCuries with efficacious response. Bone density measurement by DEXA was performed and showed Z-scores of -3.9 in the spine and -7.8 in the forearm. Noting a near-normal 25-hydroxy-vitamin D level, bisphosphonate therapy was initiated. Upon follow-up 2 months later, patient had calcium 6.6 mg/dL, alkaline phosphatase 200 U/L, and 25-hydroxy-vitamin D 7.9 ng/mL, which was confirmed by repeat testing. PTH was appropriately elevated to 382 pg/mL, phosphorus 1.9 mg/dL and 1,25-dihydroxy-vitamin D was >180 pg/mL. Bisphosphonate therapy was stopped and patient was placed on aggressive calcium and vitamin D replacement with resolution of hypocalcemia over several months. Throughout this time alkaline phosphatase has remained elevated, most recently 274 U/L. Repeat DEXA performed one year after therapy showed improvement in bone mineral density with Z-scores of -2.1 in the spine and -6.3 in forearm.

Conclusion: Hyperthyroidism is a well-known cause of secondary osteoporosis which is often mild and transient (2). Osteoporosis from hyperthyroidism may be more pronounced in cortical bone (such as forearm) compared to trabecular bone (such as lumbar spine) (3) as was seen in this patient. When patients present with more severe bone disease, as seen in this case, it is important to consider other etiologies such as a metabolic bone disease. In this case, treatment of his hyperthyroidism with resulting increase in bone mineralization likely unmasked his severe vitamin D deficiency.

(1) Kelepouris N, Harper KD, Gannon F, et al., *Ann Intern Med* 1995; 123:452.

(2) Vestergaard P, Mosekilde L, *Thyroid* 2003;13:585.

(3) Greenspan, SL, Greenspan FS, *Ann Intern Med* 1999;130:750.

Nothing to Disclose: SEF, AWG, JS

Pub # P3-129

Session Information POSTER SESSION: CLINICAL - Osteoporosis, Fractures & Bone Density (1:30 PM-3:30 PM)

Title Clinical Utility of Biochemical Markers of Bone Turnover in Pediatric Patients with Osteopenia or Osteoporosis

Author String SA Bowden, C Akusoba, JR Hayes, A Bashioum, JD Mahan
Nationwide Children's Hospital/The Ohio State University, Columbus, OH; Pacific University, Forest Grove, OR; University of North Carolina, Chapel Hill, NC; Nationwide Children's Hospital/The Ohio State University, Columbus, OH

Body

Background: The role of biochemical bone turnover markers in the care of pediatric patients with clinical bone fragility and/or low bone density is not well established. It is unclear whether these markers are useful in assessing osteoporosis and monitoring the efficacy of antiresorptive therapy in pediatric patients.

Objectives: 1) To examine the correlation of serum osteocalcin (OC) (bone formation marker) and urine pyridinoline (PD) and deoxypyridinoline (DPD) (bone resorption markers) with the bone mineral density (BMD), BMD Z scores, and other lab variables. 2) To evaluate the effects of bisphosphonates therapy on biochemical markers.

Methods: 116 patients (mean age 9.9 yrs) with clinical bone fragility and/or low BMD, (from 1998-2008), classified as osteogenesis imperfecta (41), immobilization from various neuromuscular disorders (57), juvenile osteoporosis (10), steroid-induced osteoporosis (7) were studied. 102 patients received bisphosphonates (95 pamidronate, 3 zoledronic acids, 2 risedronate, 2 alendronate). Serum OC, urine PD and DPD, other biochemical values, lumbar BMD (Hologic DEXA) with Z score (adjusted for weight and pubertal status), total bone mineral content (tBMC) at baseline and subsequent years were analyzed.

Results: There was a significant negative correlation between urine PD and lumbar BMD ($r=-0.48$, $p=0.001$), and tBMC ($r=-0.487$, $p=0.005$) at baseline. There were positive correlations between lumbar BMD, tBMC with age ($p<0.001$). Serum OC and urine PD had significantly negative correlation with age ($p<0.05$). There was no correlation between urine PD, urine DPD, serum OC and lumbar BMD Z score. There was a significant positive correlation between serum OC and urine PD ($r=0.321$, $p=0.026$), and serum alkaline phosphatase ($r=0.31$, $p=0.015$). Urine PD and DPD values and mean percentage above the normal upper limit decreased with time during bisphosphonates therapy.

Conclusions: In children with clinical bone fragility and/or low bone density, biochemical bone turnover markers were correlated with BMD, tBMC but not weight/Tanner corrected lumbar BMD Z score. While the bone turnover markers were not reliable predictors of degree of low bone density, the urine markers showed suppression during bisphosphonate therapy and may be helpful in monitoring the response to therapy.

Nothing to Disclose: SAB, CA, JRH, AB, JDM

Pub # P3-130

Session Information POSTER SESSION: CLINICAL - Osteoporosis, Fractures & Bone Density (1:30 PM-3:30 PM)

Title The Negative Effect of Increased 2-Hydroxylation of Estrogen on Femur Geometry

Author String KM Kim, KJ Kim, MH Choi, Y Rhee, S-K Lim
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Body Not only bone density, but also bone geometry is a major determinant factor for bone strength. To evaluate the effect of estrogen metabolism on bone strength in postmenopausal women, we measured femoral bone mineral densities and femoral geometry by using quantitative computed tomography and 14 types of urinary metabolites of estrogen by a gas chromatography-mass spectrometry assay system in 67 old postmenopausal women (mean age 70.2 ± 2.7 years). Among the 14 urinary metabolites of estrogen, the 2-hydroxyestrone showed negative correlations with both areal and volumetric hip BMD. Interestingly, total and cortical BMDs were significantly associated with 2-hydroxyestrone. Unlike cortical BMD, trabecular BMD did not show any significant relationship with 2-hydroxyestrone. The urinary 2-hydroxyestrone level and 2-hydroxyestrone/estrone (E1) ratio also revealed negative correlations with femur neck cortical thickness ($r=-0.415$, $p<0.05$ for 2-hydroxyestrone, $r=-0.366$, $p<0.05$ for 2-hydroxyestrone/estrone ratio), but a positive correlation with femur neck buckling ratio ($r=0.389$, $p<0.05$ for 2-hydroxyestrone, $r=0.432$, $p<0.05$ for 2-hydroxyestrone/estrone ratio). Women with a previous history of osteoporotic fracture had a higher values of 2-hydroxyestrone and 2-hydroxyestrone/estrone ratio compared with women without fracture history, but statistically no significance. Femur neck angle, femur neck width and hip axis length were not related with urinary metabolites of estrogen. In multiple linear regression analysis, waist/hip ratio was an independent determinant for level of 2-hydroxyestrone. In conclusion, the increased activity of estrogen 2-hydroxylase could accelerate the loss of bone strength, and central obesity in postmenopausal women is one of major risk factor for this derangement of estrogen metabolism.

Nothing to Disclose: KMK, KJK, MHC, YR, S-KL

Pub # P3-131

Session Information POSTER SESSION: CLINICAL - Osteoporosis, Fractures & Bone Density (1:30 PM-3:30 PM)

Title Demographics and Characteristics of Singaporean Patients with Fragility Fractures Recruited into an Osteoporosis Disease Management Program

Author String M Tan, PC Kek, RF Zhang, A Lim, M Chandran
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Body

Background and Aim: Osteoporosis is a major public health problem in Singapore. A nationwide osteoporosis disease management program (OPTIMAL- Osteoporosis Patient Targeted and Integrated Management for Active Living) aimed at preventing recurrent fragility fracture through case finding, medication subsidy, physiotherapy and case manager follow up was implemented in the 5 public hospitals in Singapore in 2008. This study aims to evaluate the baseline demographics and characteristics of patients recruited from the largest hospital.

Method: From May 2008 to February 2010, 4300 patients with osteoporotic fractures were screened at our hospital, of which, 611 patients consented to be recruited into the OPTIMAL program. This is a descriptive study and statistical analysis was performed using SPSS[reg] Statistics 17 with Fischer's Exact test for discrete variables and One-Way ANOVA for continuous variables.

Results: 546(89.4%) were females and 65(10.6%) were males. The mean age was 71 years (SD 10.2). 571 patients (93.5%) were Chinese, 26(4.3%) were Malays, 9(1.5%) were Indians and 5(0.8%) were of other ethnicity. 488 patients (79.9%) were ambulatory independent, 103(16.9%) could ambulate with walking aid and 2(0.3%) were wheelchair bound. The most common fracture type was vertebral 229(37.5%), followed by hip 143(23.4%) and distal radius 137(22.4%). Patients with distal radius fracture were younger with mean age of 64(SD 9.9) whereas patients with vertebra or neck of femur fractures tended to be older with mean age of 72(SD 9.6) and 74(SD 9.7) respectively (p=0.00). Patients who required a walking aid had more hip fractures while patients who were ambulatory independent had more distal radius fractures (p=0.00). 110 patients (18.2%) had sustained previous fractures; amongst them 40(36.4%) had a fracture at a site similar to current episode.

Conclusion: The majority were Chinese females. This could be because osteoporotic fractures are more common in Chinese females, though we should also consider that Chinese females may be more receptive to participating in structured prevention programs. A significant number of patients had history of previous fractures. This concurs with worldwide studies that show that history of previous fractures is a very significant risk for subsequent fractures. Programmes like ours that aim to prevent recurrent fragility fracture may show great promise in decreasing the burden of this devastating disease.

Nothing to Disclose: MT, PCK, RFZ, AL, MC

Pub # P3-132

Session Information POSTER SESSION: CLINICAL - Osteoporosis, Fractures & Bone Density (1:30 PM-3:30 PM)

Title Reasons for Low Prevalence of Fracture Prevention Therapy in Elderly Patients with Fractures

Author String D Spratt, A Howland, J Botler, D Belletti, L Lucas
Maine Medical Center, Portland, ME; Maine Medical Center, Portland, ME; Novartis Pharmaceuticals, East Hanover, NJ; Maine Medical Center, Portland, ME

Body Elderly patients who have already had osteoporotic fractures are at high risk for future fractures. Yet, their rate of fracture prevention therapy remains low. We surveyed 65 patients (mean age 79y) by phone 12-16 weeks after low trauma fractures to determine the rate of treatment with vitamin D, calcium, and antiresorptives and the reasons for nontreatment. Patients had low trauma hip (n=44), wrist (n=20) or spine (n=1) fractures. Questions addressed: 1) awareness of increased fracture risk and of available options to decrease the risk, 2) receptiveness to treatment, 3) specific reasons for not taking antiresorptives, and 4) preferred source of information. 17%, 63% and 81% of patients were taking an antiresorptive, calcium, and vitamin D respectively. Awareness: Only 34% of patients felt they had an increased risk of fracture while 39% thought they had a decreased risk. 45% recalled being told by their healthcare providers (HCP) that they had an increased risk. 26% remembered discussing bone health and 36% fall prevention with their doctors or nurses. Receptiveness: Only 9% felt that medications were not available to help them decrease their fracture risk. 65% felt that taking calcium and vitamin D was very important and 76% that fall prevention was very important. Reasons for nontreatment: The most common reason provided for not taking medications was no recollection of a recommendation from their HCPs. Only 5% could recall discussing antiresorptive therapy with their HCPs. Preferred source of information: More than 90% of patients preferred receiving their information during discussions with their doctors or nurses. We conclude that the low rate of fracture prevention in this high risk population is accompanied by a low rate of awareness of their risk and options for treatment but a high rate of receptivity to being treated to reduce fracture risk. Patients rely on their caregivers for information. Simple measures to facilitate orthopedists alerting the PCPs to these patients' fracture diagnosis and risk and to help PCP offices communicate with the patients may improve continuity of treatment and fracture prevention. Use of more convenient treatment regimens for this patient population such as monthly vitamin D or bisphosphonates or long-acting parenteral antiresorptives may complement these measures.

Nothing to Disclose: DS, AH, JB, DB, LL

Pub # P3-133

Session Information POSTER SESSION: CLINICAL - Osteoporosis, Fractures & Bone Density (1:30 PM-3:30 PM)

Title Osteoporosis in Men: Who Should We Treat?

Author String DC Rivera, JM Bruder, NM Mangat, JE Michalek, BD Mitchell, Y Ouyang
University of Texas Health Science Center at San Antonio, San Antonio, TX; University of Texas Health Science Center at San Antonio, San Antonio, TX; University of Texas Health Science Center at San Antonio, San Antonio, TX; University of Maryland School of Medicine, Baltimore, MD

Body Men over the age of 50 years with osteoporosis diagnosed by bone mineral density (BMD) T-score of ≤ -2.5 should be considered for pharmacological treatment. The FRAX calculator is used to estimate an individual's fracture risk probability over 10 years using the BMD at the femoral neck and various risk factors. Clinicians use this tool as a guide to determine if it would be cost effective to treat patients with T-scores between -1.0 and -2.5.

The aim of our study was to compare the treatment decision in Hispanic men ≥ 70 years of age with T-scores between -1.0 and -2.5 at the spine or hip using the FRAX calculator to the treatment decision based on a T-score at the radius of ≤ -2.5 . BMD at the spine, hip, and radius, measured as part of routine care, were reviewed in 87 Hispanic men with prostate cancer. 20 age-matched Hispanic men without prostate cancer had BMD measurements as part of the Genetics of Bone Density in Mexican-Americans Study.

There was no significant variation in the association between osteoporosis at the spine and/or hip and osteoporosis at the 1/3 radius in the two groups ($p=0.32$) and so the results were pooled ($n=107$). When using the spine and/or hip T scores ≤ -2.5 as a criteria for osteoporosis requiring pharmacological treatment, 36% ($n=38$) met the criteria. When we included the 1/3 radius, an additional 21% ($n=23$) met criteria. Therefore, when all four sites (spine, femoral neck, total hip, and 1/3 radius) were used, 57% ($n=61$) met criteria for treatment with T-scores ≤ -2.5 . This was found to be clinically significant ($p=0.004$).

In 60 men with T-scores between -1.0 and -2.5 at the spine and/or hip, 1 met criteria for pharmacologic treatment using the FRAX calculator. This is in contrast to identifying 23 additional patients needing treatment using BMD measurements at the 1/3 radius.

In Hispanic men 70 years of age or older, using the 1/3 radius T-score identifies more patients for osteoporosis treatment compared to the FRAX calculator in patients with T-scores between -1.0 and -2.5 at the spine and hip. How this translates to fracture reduction with treatment is unknown.

Nothing to Disclose: DCR, JMB, NMM, JEM, BDM, YO

Pub # P3-134

Session Information POSTER SESSION: CLINICAL - Osteoporosis, Fractures & Bone Density (1:30 PM-3:30 PM)

Title 25(OH) Vitamin D Status among Patients with Hip Fractures at San Juan De Dios Hospital in Costa Rica

Author String A Aguilar-Valdes, CH Chen-Ku
Hospital San Juan de Dios, San José, Costa Rica; University of Costa Rica, San Pedro, Costa Rica

Body

Introduction: vitamin D deficiency is prevalent in the elderly and especially in patients with hip fractures. A low vitamin D level is a risk factor for falls. Costa Rica is a tropical country where we have ultraviolet radiation exposition all year round. Low vitamin D levels have been reported previously in subjects exposed to high ultraviolet radiation such as in Hawaii (1).

Objective: to determine 25-OH vitamin D levels in patients with hip fractures at the Hospital San Juan de Dios in Costa Rica.

Materials and methods: from August to December 2010, 25 OH vitamin D levels were measured in all patients hospitalized with hip fractures at San Juan de Dios Hospital. Demographic variables were collected. Statistical analysis was performed using SPSS 18.0.

Results: 23 patients were analyzed. 56.5% were females. All patients live in Costa Rica's Central Valley. Average age was 72.01± 12.05 years (range 42.21-86.52). 56.52% were between 70 and 89 years. 52.2% had hypertension, 13% coronary artery disease, 4.3% heart failure, 26.1% had type 2 diabetes and 4.3% had type 1 diabetes. 8.7% had chronic obstructive pulmonary disease and 12.9% had malignancies. No patients were using chronic glucocorticoids or anticoagulants. 1 patient (4.3%) was using aromatase inhibitors. 47% had a transtrochanteric fracture, 26% subcapsular, and 13% transcervical. Mechanism of fracture was a fall in all patients. Mean 25 OH vitamin D levels were 19.98 ± 7.42 ng/ml (range 8.04-38.6). 56.5% of patients had levels less than 20 ng/ml, 39.1% had levels 20-30 ng/ml and only 4.3% had levels greater than 30 ng/ml. There were no differences in vitamin D levels between males and females, age or fracture site. On follow up, one patient died due to bradyarrhythmias (patient had a history of complete AV block).

Discussion: although Costa Rica is a tropical country, we previously reported several cases of vitamin D deficiency. In the present study we showed a very high prevalence of low vitamin D status in patients with hip fractures. Low vitamin D levels are a risk factor for fractures and falls. Compared to other series, Fisher et al (2) reported in Australia a prevalence of 25-OH vitamin D lower than 20 ng/ml in 77-82% of patients with hip fractures. In a series from Baltimore and Boston (3), 96% of women with hip fractures had 25 OH vitamin D of less than 32 ng/ml.

Conclusions: patients with hip fractures in Costa Rica have a high prevalence of low vitamin D status.

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Nothing to Disclose: AA-V, CHC-

Pub # P3-135

Session Information POSTER SESSION: CLINICAL - Osteoporosis, Fractures & Bone Density (1:30 PM-3:30 PM)

Title The Importance of Morphometric Radiographic Vertebral Fracture Assessment for the Detection of Patients Who Need Pharmacological Treatment: Among Postmenopausal Diabetic Korean Women

Author String YJ Choi, SJ Han, HJ Kim, S-O Yang, CS Shin, Y-S Chung
Ajou University School of Medicine, Suwon, Korea; Asia Cancer Center, Busan, Korea; Seoul National University College of Medicine, Seoul, Korea

Body

Purpose: Vertebral fracture assessment (VFA) is important for identifying patients that need pharmacologic therapy. However, many patients with VFs remain undiagnosed and untreated. This study evaluated the number of patients that would be candidates for treatment by the National Osteoporosis Foundation (NOF) Clinician's Guidelines to the Prevention and Treatment of Osteoporosis, among postmenopausal diabetic Korean women with morphometric VFs, assumed to have no radiographic diagnosis of VFs.

Methods: A total of 873 postmenopausal diabetic women were enrolled. Lateral plain radiographs of the thoracolumbar spine, and total hip BMD were obtained. The FRAX[reg] probability was computed using the algorithm available online at <http://www.shef.ac.uk/FRAX> (South Korea version). The subjects were classified with and without VFs into candidates for treatment [Tx(+)] or no treatment [Tx(-)] according to the NOF pharmacologic treatment guidelines, regardless of the presence of VFs.

Results: Among the subjects with morphometric VFs, only 2% of the patients had previously diagnosed VFs by physicians. There were 33.6% of the patients with current back pain that had VFs. However, 73.6% of the patients with VFs were not included in the Tx(+) group, given the assumption of no radiographic diagnosis of VFs.

Conclusions: With regard to increased risk of VFs in postmenopausal Korean women with type 2 DM, radiographic VFA would be useful for the clinical identification of osteoporosis and fractures.

Nothing to Disclose: YJC, SJH, HJK, S-OY, CSS, Y-SC

Pub # P3-136

Session Information POSTER SESSION: CLINICAL - Osteoporosis, Fractures & Bone Density (1:30 PM-3:30 PM)

Title The High Prevalence of Morphometric Vertebral Fracture Is Independent of Bone Mineral Density in Postmenopausal Korean Diabetic Patients

Author String YJ Choi, SJ Han, HJ Kim, DJ Chung, S-O Yang, CS Shin, SK Lim, Y-S Chung
Ajou University School of Medicine, Suwon, Korea; Chonnam National University College of Medicine, Gwangju, Korea; Asia Cancer Center, Busan, Korea; Seoul National University College of Medicine, Seoul, Korea; Yonsei University College of Medicine, Seoul, Korea

Body

Objective: An increased risk of fracture in patients with diabetes mellitus (DM) compared to general population has been reported, but its association to bone mineral density (BMD) is unclear. The aim of the study was to evaluate the effect of BMD on the prevalence of osteoporotic vertebral fractures (VFs) in postmenopausal Korean diabetic patients.

Methods: This was an observational, non-randomized study performed at medical centers. Among 2239 subjects recruited, a total of 873 postmenopausal diabetic patients were enrolled and lateral radiographs of the thoracic and lumbar spine and total hip BMD values were obtained.

Results: Of the 873 subjects who underwent hip BMD measurements, 402 women (46%) had VFs. There was no significant difference in hip BMD between those with and without VFs after adjustment for age. The prevalence of VFs was similar in all T-score ranges, but the absolute number of patients with VFs was the highest for T-scores ranging from -1.0 to -1.5. The BMD value was not identified as a risk factor for VFs in postmenopausal diabetic patients by logistic regression analyses.

Conclusion: Our findings indicate that VF rate is not associated to BMD in postmenopausal type 2 DM Korean subjects. Therefore, BMD might be an inappropriate diagnostic tool for detecting VFs in postmenopausal diabetic patients. Since, bone strength is a reflection of BMD and bone quality; diagnostic tools that can evaluate bone quality are required to prevent fragility fractures in DM patients.

Nothing to Disclose: YJC, SJH, HJK, DJC, S-OY, CSS, SKL, Y-SC

Pub # P3-137

Session Information POSTER SESSION: CLINICAL - Osteoporosis, Fractures & Bone Density (1:30 PM-3:30 PM)

Title Two Unusual Cases of Osteoporosis in Young Male Patients

Author String A Fusco, A Bianchi, D Iacovazzo, F Veltri, M Mormando, S Piacentini, M Lorusso, G Gambaro, L De Marinis
Catholic University School of Medicine, Rome, Italy; Catholic University School of Medicine, Rome, Italy

Body Osteoporosis in young male patients is an uncommon finding. We report two cases of male patients who came to our attention for evaluating a condition of osteoporosis in a young age. The first patient, aged 48, presented with osteoporosis (lumbar spine T-score -2.5); vitamin D deficiency, hyperparathyroidism and other common causes were excluded. He referred to suffer from recurrent dental abscesses since a long time and his brother seemed to suffer from the same problems. Bone alkaline phosphatase was found to be elevated. Biochemical testing revealed low plasma phosphate levels and urinary phosphate sampling showed an inappropriately low phosphate reabsorption rate (73%). Diagnosis of hypophosphatemic rickets was done, despite the absence of other clinical and radiological findings. The patient underwent dosing of FGF-23, fibroblast growth factor-23, a molecule that modulates kidney phosphate homeostasis; plasma dosing came in the normal range, thus to exclude genetic mutations in PHEX and FGF-23 genes whose function directly correlates with FGF-23 plasma levels. The mutational analysis for other specific genes involved in phosphate homeostasis, NHERF-1, NPT2a and NPT2c, is now ongoing. The second patient, aged 30, had a familiar history of severe osteoporosis not responsive to anabolic therapy. He underwent a bone densitometry that revealed a condition of osteoporosis (lumbar spine T-score -2.7). Common causes of secondary osteoporosis were excluded. Due to concomitant hypercalciuria and proteinuria he was evaluated by a nephrologist and underwent an ammonium chloride acidification test that was diagnostic for type 1 renal tubular acidosis. Osteoporosis in young male patients with no other common evident causes can be a manifestation of rare kidney diseases on a genetic base. A prompt diagnosis in these cases can be helpful in starting specific treatments and allow to begin an appropriate follow up, thus to prevent, in specific cases, the evolution to chronic kidney disease.

Nothing to Disclose: AF, AB, DI, FV, MM, SP, ML, GG, LDM

Pub # P3-138

Session Information POSTER SESSION: CLINICAL - Osteoporosis, Fractures & Bone Density (1:30 PM-3:30 PM)

Title Vitamin D Status and Fractures in Liver Transplant Patients: Long-Term Data

Author String E Segal, Y Baruch, M Nodelman, S Ish-Shalom
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Body Osteoporosis represents a serious problem in liver transplant patients. Ten years ago we evaluated 29 liver transplant patients. Nineteen (65.5%) had decreased bone mass, 11 (37.8%) were osteoporotic, 28 (96.5%) had serum level of 25(OH)D<20 ng/ml, mean 12.52±3.19, mean PTH 59.67 ng/l±29.78. The patients received letters containing the diagnosis and treatment recommendations. Now we re-evaluated charts of all those patients'. 7 patients died, no one of the 7 sustained an osteoporotic fracture. One patient with renal failure was excluded from the analysis. 10 men, 10 women (postmenopausal), age 58.8±15.8. Fifteen fractures were reported following transplantation, 10 (75%) within the first year, none in the last ten years. Post-transplant time was 18 ±5.2 years. 7 (35%) received cyclosporine A, 13 (65%) tacrolimus, 6 (30%) were currently on glucocorticoid therapy with prednisone 5-10 mg/d. Fifteen patients (75%) received 600-1200 mg of elemental calcium, and 400-1600 IU of vitamin D daily. Four (20%) patients were also treated with bisphosphonates. PTH levels ranged 29-71 ng/l, 25OHD levels- 14-42 ng/ml. Mean PTH 53.12±42, p=0.6; mean 25OHD 28.2±7.58, p=0.002 compared to the first evaluation. There was no difference in 25OHD in Cyclosporin A and Tacrolimus patients: 26.2±9.8 and 28.1±7.1, respectively. BMD results of 9 patients were available, none of them shown significant changes compared to the first evaluation. **Conclusion:** Majority of the liver transplant patients continued with calcium and Vitamin D supplementation. Sufficient vitamin D level, and increased post- transplantat time might have positive effect on patients' metabolic status and fractures rate.

Nothing to Disclose: ES, YB, MN, SI-S

Pub # P3-139

Session Information POSTER SESSION: CLINICAL - Osteoporosis, Fractures & Bone Density (1:30 PM-3:30 PM)

Title Relationship between Bone Formation Markers Bone Alkaline Phosphatase, Osteocalcin and Amino-Termina Propeptide of Type I Collagen, and Bone Mineral Density in Elderly Men: Preliminary Results

Author String F Lumachi, SMM Basso, P Cappelletti, R Orlando
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Body

Background: Bone formation and resorption are altered in all metabolic bone diseases, especially in postmenopausal women. To predict changes in bone mineral density (BMD) is useful to manage the progression of the disease, and potentially to provide interventions in reducing fracture risk. With the aim of monitoring these mechanisms, a number of serum markers have been studied. Bone alkaline phosphatase (BAP) is widely used in studying metastatic bone disease, while osteocalcin, which is mainly synthesized by osteoblasts, is an useful marker whenever resorption and formation are uncoupled. During processing of collagen I, before the fibril formation, there is a cleavage of the aminoterminal extension peptide, named amino-terminal propeptide of type I collagen (PINP), which represents another serum marker of bone formation. The aim of this study was to evaluate the relationship between BAP, osteocalcin, PINP and BMD in elderly (>65 years) men.

Patients and Methods: Serum BAP, osteocalcin, and PINP were measured in duplicate in a group of 18 elderly (69.5±5.4 years) men, who also underwent lumbar-spine (L2-L4) dual-energy x-ray absorptiometry and BMD measurement. Patients with hypogonadism, renal or liver diseases, hyperparathyroidism, cancer, or those on any therapy affecting bone (i.e. corticosteroids, vitamin D, calcium, calcitonin) were excluded from the study.

Results: The mean BMD and body mass index (BMI) were 0.963±0.04 g/cm² and 24.4±1.2 kg/m², while BAP, osteocalcin and PINP serum levels were 27.8±11.3 U/L, 25.6±7.1 ng/mL and 36.0±7.5 ng/mL, respectively. No correlation was found between BMD and BAP (R=-0.28, p=0.25), osteocalcin (R=-0.18, p=0.48) and PINP (R=-0.21, p=0.39). There was no correlation between BMI and both age (R=0.05, p=0.83) and BMD (R=0.10, p=0.67). Only a significant relationship between age and PINP (R=-0.52, p=0.02) was observed.

Conclusions: In this group of patients we did not find any relationship between bone serum markers and BMD, and moreover all of them seem to be an independent parameter. Thus, they are not useful in monitoring bone mineral status of elderly men.

Lumachi F et al., Aging Male 2009; 12: 144

Nothing to Disclose: FL, SMMB, PC, RO

Pub # P3-140

Session Information POSTER SESSION: CLINICAL - Osteoporosis, Fractures & Bone Density (1:30 PM-3:30 PM)

Title The Relation between Bone Mineral Density and Blood Pressure Values: The Iranian Multi-Center Osteoporosis Study (IMOS)

Author String HR Aghaei Meybodi, P Khashayar, M Abbasalizad Farhangi, M Rezaei Homami, A Hossein-Nezhad, R Heshmat, B Larijani
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Body Background- Epidemiological evidences suggest bone mineral density (BMD) values to be associated with variations in blood pressure levels. Considering the limitations of the human studies previously published in this field, including the small number of the participants without adjusting for confounding variables, the present study was conducted to investigate the relationship between BMD and blood pressure values in an Iranian population.
Methods- The study population comprised of apparently healthy men and women who participated in the Iranian Multi-centric Osteoporosis Studies (IMOS), a cross-sectional study carried out in several urban areas of the country. The blood pressure measures and bone mineral density (BMD) values at different sites were recorded. The influence of BMD values on systolic and diastolic blood pressure was assessed based on a list-wise method.
Results- In univariate analysis, a significant negative relationship was found between DBP and BMD values at the femoral neck ($r = -0.11$, $P < 0.001$) and total hip ($r = -0.08$, $P = 0.01$) among men. In female participants, there was a similar correlation between DBP and BMD values at the femoral neck ($r = -0.12$, $P < 0.001$), total hip ($r = -0.06$, $P = 0.04$) and lumbar spine ($r = -0.115$, $P < 0.001$). SBP was negatively correlated with BMD values at the femoral neck ($r = -0.196$, $P < 0.001$) and lumbar spine ($r = -0.208$, $P < 0.001$) in women. After adjusting for age, body mass index, serum calcium levels and PTH, neither of these associations remained significant in either gender.
Conclusion- There is no significant association between blood pressure levels and BMD values after controlling for certain confounders was observed.

Nothing to Disclose: HRAM, PK, MAF, MRH, AH-N, RH, BL

Pub # P3-141

Session Information POSTER SESSION: BASIC - Reproductive Neuroendocrinology (1:30 PM-3:30 PM)

Title Glucocorticoids Inhibit Kisspeptin Neurons during Stress-Induced Reproductive Inhibition

Author String O Wang, A Lanjuin, J Basko, C Dulac, J Majzoub
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Body Stress responses are designed to improve the chances of immediate survival at the expense of nonessential bodily functions, including reproduction. Though stress is a potent inhibitor of the hypothalamic-pituitary-gonadal (HPG) axis, the molecular mechanisms by which stressors target this axis are unknown. Kisspeptin, encoded by the *Kiss1* gene, is a global regulator of the HPG axis. We hypothesize that stress, via the murine stress hormone corticosterone, regulates *Kiss1* expression to inhibit downstream reproductive function. Adult male mice were subjected to 5h of restraint or 48h of food deprivation. Plasma was collected for hormone assays, and *Kiss1* expression in the arcuate nucleus was quantified by an automated macro using NIH ImageJ after *in situ* hybridization. Restrained animals had higher corticosterone (40.6 ± 1.4 vs. 2.7 ± 0.9 [micro]g/dL, $p < 0.01$), lower luteinizing hormone (LH) (0.03 ± 0.01 vs. 0.19 ± 0.07 ng/mL, $p = 0.08$), and lower testosterone (T) levels (0.03 ± 0.01 vs. 4.0 ± 1.7 ng/mL, $p < 0.05$) compared to controls. Food-deprived mice also had higher corticosterone (40.6 ± 2.3 vs. 2.9 ± 0.5 [micro]g/dL, $p < 0.01$), lower LH (0.02 ± 0.001 vs. 1.1 ± 0.5 ng/mL, $p = 0.05$), and lower T levels (0.09 ± 0.01 vs. 6.6 ± 2.7 ng/mL, $p < 0.05$) compared to controls. Restrained mice had fewer *Kiss1* mRNA-positive cells compared to unrestrained controls (151.7 ± 45.3 vs. 344.6 ± 38.7 cells, $p < 0.05$), and food-deprived mice had fewer *Kiss1* mRNA-positive cells compared to fed controls (67.3 ± 20.2 vs. 379.8 ± 75.5 cells, $p < 0.05$). Consistent with the hypothesis that kisspeptin neurons respond to glucocorticoids, glucocorticoid receptors were identified in kisspeptin neurons. Additionally, mice injected with 40mg/kg corticosterone i.p. had lower LH (0.13 ± 0.02 vs. 0.74 ± 0.53 ng/mL, $p = 0.29$), lower T (0.09 ± 0.02 vs. 4.2 ± 2.0 ng/mL, $p < 0.01$), and fewer *Kiss1* mRNA-positive cells compared to saline-injected controls (193.8 ± 51.5 vs. 371.4 ± 74.3 cells, $p < 0.05$). Thus, under all high corticosterone conditions tested (restraint, food deprivation, i.p. corticosterone), *Kiss1* expression was decreased. We are currently examining whether glucocorticoids directly inhibit kisspeptin neurons by using mice with kisspeptin neuron-specific deletion of glucocorticoid receptors (*Kiss1*^{CreBAC} *GR*^{flox/flox}). We hypothesize that the reproductive axis in these mice will be resistant to stress-induced inhibition.

Sources of Research Support: National Science Foundation, Graduate Research Fellowship; Department of Defense, National Defense Science and Engineering Graduate Fellowship.

Nothing to Disclose: OW, AL, JB, CD, JM

Pub # P3-142

Session Information POSTER SESSION: BASIC - Reproductive Neuroendocrinology (1:30 PM-3:30 PM)

Title Sexually Dimorphic Testosterone Secretion in Prenatal and Postnatal Mice Is Independent of Kisspeptin-Kiss1r Signaling

Author String MC Poling, AS Kauffman
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Body Kisspeptin, a neuropeptide encoded by the *Kiss1* gene, is a potent secretagogue of GnRH, and is therefore critical for sex steroid secretion in adulthood. However, kisspeptin's role in regulating sex steroid secretion during perinatal development is unexplored. In mice, testosterone (T) levels are higher in males than females several days before birth and again later during the first five hours after birth. Evidence suggests that prenatal gonadal T secretion may occur independently of GnRH influence; however, whether the elevated T in postnatal day 1 (P1) male mice is governed by GnRH neurons or upstream kisspeptin circuits is unknown. In Exp. 1, we determined the role of kisspeptin-Kiss1r signaling in P1 T secretion. Blood and brains were collected either within the first 4 hours after birth (4h group; when T is normally elevated in males but not females) or 16-20 hours after birth (20h group) in P1 *Kiss1r* KO and wildtype (WT) mice. First, we used *in situ* hybridization to determine if higher T levels in 4h WT males than females might be driven by higher upstream *Kiss1* levels in the brains of males. Interestingly, there was no difference in hypothalamic *Kiss1* expression between 4h and 20h males, and males did not have higher *Kiss1* levels than females at either time-point. Next, we measured serum T levels in P1 mice of both genotypes. As expected, 4h WT males had higher T levels than 4h WT females. Surprisingly, 4h *Kiss1r* KO males also had higher T levels than 4h females, indicating that P1 males retain their ability to secrete T in the absence of kisspeptin signaling. Next, in Exp. 2, we tested whether kisspeptin-Kiss1r signaling is required for prenatal T secretion. We used *in situ* hybridization to examine hypothalamic progesterone receptor (*Pgr*) expression, a well-established proxy for T levels in prenatal rodents (higher T levels in embryonic males promote hypothalamic *Pgr* expression). Interestingly, we found no difference in hypothalamic *Pgr* expression between embryonic day 18 (E18) *Kiss1* KO and WT males, both of which had much higher *Pgr* levels than E18 females. This suggests that *Kiss1r* KO males retain the ability to secrete elevated T during late embryonic life. We conclude that, unlike in adulthood, elevated prenatal and postnatal T secretion in mice is likely independent of kisspeptin signaling. Whether elevated perinatal T secretion is also independent of GnRH signaling is currently being determined.

Sources of Research Support: NIH grants R01 HD065856, R00 HD056157, and U54 HD012303.

Nothing to Disclose: MCP, ASK

Pub # P3-143

Session Information POSTER SESSION: BASIC - Reproductive Neuroendocrinology (1:30 PM-3:30 PM)

Title Differential Negative Feedback Responses of Neurokinin B and Kisspeptin Expression Indicate Sensitivity Differences to Estrogen in the Arcuate Nucleus

Author String JC Gill, C Martin, C Kwong, RS Carroll, UB Kaiser
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Body Growing evidence indicates a collaborative neuroendocrine role of neurokinin B (NKB) and kisspeptin (Kiss1) in gonadotropin secretion and reproductive function, though the precise interplay is unclear. NKB is a potential modulator of Kiss1 activity and GnRH secretion, but contradictory evidence exists regarding the contribution of the steroid hormone milieu to NKB function in reproduction. NKB and Kiss1 are co-expressed within neurons of the arcuate nucleus (ARC), a hypothalamic site important for negative feedback regulation by gonadal sex steroids. We have shown previously developmental patterns of mouse ARC NKB and Kiss1 expression that suggested differences in sex steroid negative feedback. To test the hypothesis that Kiss1 expression is more sensitive to estrogen (E2) negative feedback than NKB, functional studies were conducted *in vivo*. In female mice, Silastic capsules with three E2 doses (vehicle control (Veh), Low and High) were implanted for 7 days in ovariectomized (OVX) adult (P60) WT, which achieved physiological ranges of E2. Juvenile (P13) and adult (P60) hypogonadal *hpg* mice, deficient in GnRH, were also treated in this manner. Responses to E2 treatments were confirmed by corresponding increases in uterine weights. On day 7, ARC tissues from OVX Adult WT, Adult *hpg* and Juvenile *hpg* (n=10-13) were processed for quantitative RT-PCR. All groups displayed a robust negative feedback response that at **Low E2 significantly suppressed Kiss1 expression** (% change from Veh: OVX Adult WT -68%, Adult *hpg* -57% and Juvenile *hpg* -48%; P<0.05). Kiss1 expression was further repressed by High E2 (% change from Veh: -81%, -92% and -92%, respectively). In contrast, **NKB expression was significantly suppressed only by High E2** (% change from Veh: OVX Adult WT -33%, Adult *hpg* -47% and Juvenile *hpg*, -48%; P<0.05). The NKB response to E2 was similar between Juvenile and Adult *hpg*, suggesting that negative feedback is developmentally intact at prepubertal ages, but that higher levels of E2 are necessary to manifest E2 negative feedback suppression of NKB. These findings provide evidence of differential regulation of NKB and Kiss1 in the ARC by E2, and may represent a mechanism that distinguishes NKB from Kiss1 contributions in puberty and reproductive function. Importantly, these results suggest that ARC Kiss1 and NKB regulation by E2 would establish distinct patterns of activation and repression across the estrous cycle.

Nothing to Disclose: JCG, CM, CK, RSC, UBK

Pub # P3-144

Session Information POSTER SESSION: BASIC - Reproductive Neuroendocrinology (1:30 PM-3:30 PM)

Title Morphological Alterations in KNDy-GnRH Neurocircuitry in a Sheep Model of PCOS

Author String M Cernea, V Padmanabhan, L Coolen, C Merkley, L Jackson, T Lee, M Lehman
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Body Polycystic ovarian syndrome (PCOS) is a common reproductive disorder, whose symptoms include altered sensitivity to steroid feedback control of gonadotropin releasing hormone (GnRH) secretion. Recent evidence suggests that a subset of neurons in the arcuate nucleus (KNDy cells), that co-express the neuropeptides, kisspeptin, neurokinin B, and dynorphin, and the vesicular glutamate transporter 2 (VGlut2), plays a critical role in steroid feedback regulation of GnRH. In addition, KNDy cells form a reciprocally connected network, hypothesized to be critical for generating GnRH pulses. Prenatal testosterone (T)-treated ewes show reproductive deficits similar to PCOS women and moreover show changes in KNDy neuropeptide expression. However, it is currently unknown if prenatal T exposure results in changes in the reciprocal connections that exist among KNDy cells, their projections to GnRH cells, and somal size of KNDy cells.

To address this question, control (n=5) and prenatal T-treated (n=4) adult ewes were ovariectomized and received estradiol implants (OVX+E) designed to mimic follicular phase levels of estradiol. Reciprocal connections between KNDy cells were visualized using immunocytochemistry for kisspeptin/vGlut2/synaptophysin, and KNDy cell connections to GnRH neurons with staining for kisspeptin/vGlut2/GnRH. Confocal analysis was conducted. Prenatal T-treated animals showed a significant decrease ($P<0.05$) in the number of reciprocal (kisspeptin/vGLUT₂ dual) inputs and total synaptic inputs onto KNDy cells. In addition, fewer KNDy projections (kisspeptin/vGLUT₂ dual inputs) on GnRH soma in the mediobasal hypothalamus and preoptic area were detected. These decreases in reciprocal connections among KNDy neurons, and their projections onto GnRH neurons, may contribute to defects in the ability of KNDy cells to mediate steroid feedback control of GnRH secretion. Analysis of the somal size of KNDy cells revealed a significant increase ($P<0.05$) in prenatal T-treated ewes, similar to that previously reported for arcuate kisspeptin cells in the postmenopausal female human brain. This increase may be a compensatory response to decreased synaptic input. Overall, prenatal T treatment caused morphological changes and loss of reciprocal connections within the KNDy population, and their projections to GnRH neurons. Together these alterations may contribute to the defects in steroid feedback control of GnRH secretion seen in prenatal T-treated ewes.

Sources of Research Support: NIH P01 HD044232 to V.P. and M.N.L.

Nothing to Disclose: MC, VP, LC, CM, LJ, TL, ML

Pub # P3-145

Session Information POSTER SESSION: BASIC - Reproductive Neuroendocrinology (1:30 PM-3:30 PM)

Title Kisspeptin Cells in the Ovine Arcuate Nucleus (ARC) Express Prolactin Receptors but Not Melatonin Receptors

Author String Q Li, IJ Clarke, JT Smith
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Body Many species, including sheep, are seasonal breeders and the dominant role of photoperiod in the control of reproduction is well established. Melatonin, secreted at night by the pineal gland, transforms photoperiod to a humoral signal that regulates brain systems controlling reproduction. The mechanism by which melatonin regulates reproduction is not known. The circannual rhythmicity of a number of factors including prolactin, is also regulated by photoperiod through changes in melatonin secretion. Plasma prolactin levels in sheep are higher during the non-breeding season. Kisspeptin is synthesised from neurons in the ovine arcuate nucleus (ARC) and preoptic area and stimulates the hypothalamic-pituitary-gonadal (HPG) axis, mediating sex steroid feedback control of gonadotropin releasing hormone (GnRH) secretion. Kisspeptin gene expression in the ARC is lower in the non-breeding season, so we hypothesized that this is directly, or indirectly, regulated by melatonin and/or prolactin. First, we examined the expression of melatonin receptor (MT1) in kisspeptin neurons in the ARC of ovariectomised (OVX) sheep using double-label in situ hybridization (ISH). Kisspeptin neurons were identified by digoxigenin-labelled riboprobes and MT1 neurons by radioactive 35S-labelled riboprobes. No MT1 receptor expression was found on kisspeptin neurons, while strong MT1 mRNA expression was confirmed in the pars tuberalis. We then measured expression of the long-form prolactin receptor (prLR) in ARC kisspeptin neurons of ovariectomised ewes during the breeding and non-breeding seasons, using double-label ISH. From a total of 992 kisspeptin neurons examined in 3 animals during the breeding season, 62±8% co-expressed prLR mRNA. Similarly, in 1111 kisspeptin neurons examined in 4 animals in the non-breeding season, 62±5% co-expressed prLR mRNA. Thus, ARC kisspeptin cells do not express MT1, but do express prLR, which may allow direct response to seasonal changes in prolactin levels. There was no seasonal change in prLR in kisspeptin neurons. Studies are in progress to determine whether prolactin treatment during the breeding season (when prolactin levels are low) regulates kisspeptin expression in the ARC.

Sources of Research Support: Australian NHMRC.

Nothing to Disclose: QL, IJC, JTS

Pub # P3-146

Session Information POSTER SESSION: BASIC - Reproductive Neuroendocrinology (1:30 PM-3:30 PM)

Title Time of Puberty Onset Is Regulated by Estrogen Signaling in Kisspeptin Neurons

Author String A Lanjuin, C Dulac
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Body In mice, as in humans, puberty onset occurs within a restricted time window and is initiated by the [ldquo]awakening[rdquo] of the Hypothalamic-pituitary-gonadal (HPG) axis, a signaling loop that links neuronal activity in the hypothalamus to the secretion of gonadotropins from the pituitary and stimulation of sex hormone production from the gonads. Restraint of this axis during juvenile stages is required to prevent premature sexual function, and has been shown to involve both sex hormone dependent and independent mechanisms. The neuropeptide Kisspeptin is a potent regulator of HPG axis function in adults, and a critical determinant of puberty onset. Importantly, Kisspeptin expression is subject to complex regulation by sex hormones, and could thus mediate the integration of sex hormone dependent and independent influences on puberty.

To directly determine the contribution of Estrogen signaling within Kisspeptin neurons to the control of event at puberty, we generated a mouse line in which Kisspeptin expressing neurons are made insensitive to Estrogen Receptor alpha mediated inputs. This was achieved by genetic ablation of Estrogen Receptor alpha ($ER\alpha$) specifically in Kiss1-expressing neurons (Kiss1::Cre; $ER\alpha$ flox/flox). We find that Kiss1::Cre; $ER\alpha$ flox/flox females exhibit a dramatic advance in puberty onset, a full 8 days earlier than their control littermates as measured by multiple markers of puberty progression. Our findings provide strong support to the idea that Kisspeptin neurons mediate Estrogen dependent negative feedback effects on puberty. In addition, we show that precocious puberty onset in Kiss1::Cre; $ER\alpha$ flox/flox mice is female-specific, suggesting that distinct mechanisms control puberty onset in males and females. This dramatic phenotype provides an entry towards a more complete understanding of the complex mechanism by which timing of puberty onset is controlled.

Nothing to Disclose: AL, CD

Pub # P3-147

Session Information POSTER SESSION: BASIC - Reproductive Neuroendocrinology (1:30 PM-3:30 PM)

Title Prenatal Testosterone Excess Decreases Neurokinin 3 Receptor (NK3R) and Its Colocalization with Kisspeptin in the Ovine Arcuate Nucleus

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Body Neurokinin B (NKB; or tachykinin 3 (TAC3)), and its receptor NK3R (or TAC3R), have been the focus of recent attention in the neuroendocrine control of mammalian reproduction. In the arcuate nucleus (ARC), NKB is colocalized with kisspeptin and dynorphin in a single population (KNDy neurons: kisspeptin, NKB, dynorphin) that is proposed to serve as a final common pathway mediating steroid feedback control of GnRH secretion (1). Alterations in the balance of neuropeptide expression within KNDy cells may underlie feedback deficits in polycystic ovarian syndrome (PCOS). For example, in the prenatal testosterone (T) treated ewe, a well-characterized animal model of PCOS, there is a marked decrease in NKB peptide expression specifically within kisspeptin cells of the ARC. NKB may act as an autoregulatory transmitter in KNDy cells, since NK3R and NKB are colocalized in the same neurons, and these cells form reciprocal contacts with each other. We hypothesized that NK3R expression may be altered in the brains of prenatal T animals, and evaluated the number of the NK3R-immunopositive neurons, and the colocalization of NK3R with kisspeptin, in the hypothalami of ovariectomized control and prenatal-T animals bearing estradiol (E2) implants producing follicular phase levels of E2. The total number of NK3R-positive cells was significantly reduced in middle and caudal ARC of prenatal T-treated ewes compared to control animals; in contrast, no significant differences were observed in any other hypothalamic area containing NK3R-expressing cells, including the preoptic area (POA). As expected, in control animals, NK3R was colocalized in a majority (51.10%) of kisspeptin cells of the ARC, whereas very few kisspeptin cells in the POA (5.26 %) colocalized NK3R. Prenatal T treatment did not affect the number of kisspeptin cells of the ARC, but significantly decreased the percentage that colocalized NK3R (51.10±5.12; 30.12±3.2). Since KNDy cells comprise an interconnected network of steroid-responsive cells, and NKB is stimulatory in sheep, decreases in both NKB and NK3R in prenatal T-treated animals may impair communication among KNDy cells and their ability to convey steroid feedback signals to GnRH neurons. If so, NK3R antagonists should mimic at least some the feedback deficits seen in prenatal T-treated animals, and, conversely, NKB agonists may have potential beneficial effects in reversing reproductive neuroendocrine deficits seen in this animal model of PCOS.

(1) Lehman et al., Endocrinology, 2010

Sources of Research Support: NIH P01 HD044232 to V.P. and M.N.L.

Nothing to Disclose: TA, LMC, VP, TL, MNL, LJ

Pub # P3-148
Session Information POSTER SESSION: BASIC - Reproductive Neuroendocrinology (1:30 PM-3:30 PM)
Title Testosterone Depletion and Its Possible Mechanism of Action by Chronic Administration of Metastin Analogs, KiSS1-305, TAK-448, and TAK-683, in Adult Male Rats
Author String H Matsui, A Tanaka, K Yokoyama, Y Takatsu, K Ishikawa, T Asami, N Nishizawa, A Suzuki, S Kumano, M Terada, M Kusaka, C Kitada, T Ohtaki
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Body Metastin/kisspeptin functions as a key regulator in the control of hypothalamic gonadotropin-releasing hormone (GnRH) neurons. We previously showed that chronic administration of our metastin analogues, KiSS1-305, TAK-448, and TAK-683, depleted plasma testosterone (T) levels in male rats, dogs, and monkey (1-3). The aim of this study is to clarify the possible mechanism of action for T-lowering effect using male rats. Initiation of the chronic administration of KiSS1-305 led transient c-Fos expressions in the GnRH neurons associated with elevations of plasma luteinizing hormone (LH) and T levels, suggesting the activation of GnRH neurons and marked GnRH release. c-Fos expressions were then disappeared and these hormone levels were suppressed close to or below the detection limit (0.08 ng/mL for LH, 0.04 ng/mL for T) by day 6. Plasma LH levels were elevated in response to a GnRH analogue leuprolide acetate, but not to KiSS1-305, after 6-day chronic administration of KiSS1-305, suggesting that chronic administration of metastin analogue impaired the HPG axis at the upstream of the pituitary. We then found that single bolus injection of KiSS1-305 or TAK-683 after 6-day or 8-week chronic administration obviously induced c-Fos in the majority of GnRH neurons. The result indicates that the chronic administration of metastin analogues attenuates but does not abolish GnRH neuron's responsiveness to the peptide at least in terms of c-Fos induction. We also tested the chronic administration up to 4 weeks, and found reduction of hypothalamic GnRH contents, probably causing a blunted LH release in spite of the c-Fos induction on the injection of KiSS1-305 after 6-day administration. Since GnRH mRNA levels were unchanged after the chronic administration, the most plausible explanation is that GnRH is continuously released so that the neurons cannot recover GnRH storage. These results have led us to propose the hypothesis that chronic administration of metastin analogues decreases the responsiveness of GnRH neurons which shuts down the endogenous input, and stimulates weakened GnRH neurons to release GnRH continuously at a very low level which is incapable of stimulating the pituitary. These result in the reduction of hypothalamic GnRH contents and suppression of the HPG functions. Pharmacological potential of metastin analogues holds promise as a novel therapeutic approach for several hormone-related diseases such as prostate cancer.

- (1) Matsui H et al. 21st Meeting of the European Association for Cancer Research, 2010; Oslo, Norway
- (2) Tanaka A et al. The 22nd EORTC-NCI-AACR symposium on Molecular Targets and Cancer Therapeutics, 2010; Berlin, Germany.
- (3) Asami T et al. 5th International Peptide Symposium, 2010; Kyoto, Japan

Nothing to Disclose: HM, AT, KY, YT, KI, TA, NN, AS, SK, MT, MK, CK, TO

Pub # P3-149

Session Information POSTER SESSION: BASIC - Reproductive Neuroendocrinology (1:30 PM-3:30 PM)

Title Changes in Hypothalamic Gene Expression during Natural Reproductive Aging in Female Rats

Author String BA Kermath, DM Walker, PD Riha, AC Gore
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Body The complex mechanisms underlying menopause, and the particular role of the hypothalamus in its etiology, are unclear. The purpose of this experiment was to characterize neuroendocrine mechanisms that underlie the transition to acyclicity in a middle-aged female rat model of natural reproductive senescence by examining changes in gene expression in selected hypothalamic nuclei. We used rats (~1 year of age) that were regularly cycling (MA-Reg), irregularly cycling (MA-Irreg), or acyclic (in persistent estrus; MA-PE) to assess our hypothesis that natural reproductive decline at middle age is associated with changes in neuroendocrine gene expression. Using low-density real-time PCR arrays, 48 neuroendocrine genes that modulate GnRH release and reproductive function were quantified in 3 hypothalamic nuclei: the anteroventral periventricular nucleus (AVPV), the site of steroid positive feedback onto GnRH neurons; the arcuate nucleus (ARC), the site of negative feedback; and the median eminence (ME), the site of GnRH neurosecretion. Our preliminary data (n=6) showed surprisingly few gene expression differences in the AVPV and ARC. In the ARC, transcription factor Stat5b expression in MA-Reg females was significantly higher than in MA-PE or MA-Irreg rats, but other gene expression differences were not detectable. Also surprisingly, the ME had the greatest number of gene expression differences at $p < 0.05$. The ME showed increased gene expression for the progesterone receptor (PR), membrane PR, TGF α , and ER β in MA-Reg rats compared to the other groups. The ME of MA-Irreg rats had trends for increased kisspeptin receptor compared to other groups and showed decreased TGF β 1 gene expression compared to MA-PE rats. As there are few neuronal cell bodies in the ME, these changes are likely occurring on glial cells or nerve terminals. The few changes in gene expression in the AVPV and ARC suggest that post-transcriptional mechanisms such as protein expression may be more important targets of modulation during reproductive aging in intact female rats. Overall, the preliminary data show some changes in gene expression during the transition to reproductive senescence in the ME. Considering our recent reports for substantial structural changes in nerve terminals and glia in the aging rat ME [Yin et al. 2009 (J Comp Neurol, Endocrinology)], this is a brain area that merits future research in the context of reproductive aging.

Sources of Research Support: NIH 5RO1 AG028051.

Nothing to Disclose: BAK, DMW, PDR, ACG

Pub # P3-150

Session Information POSTER SESSION: BASIC - Reproductive Neuroendocrinology (1:30 PM-3:30 PM)

Title Differential Responses to Kisspeptin in Women in the Follicular and Luteal Phases of the Menstrual Cycle

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Body *Background:* Kisspeptin is a potent stimulus for GnRH-mediated LH secretion in all mammalian species tested to date, including humans. We have shown previously that, in healthy adult men, a single intravenous dose of kisspeptin 112-121 0.24 nmol/kg not only induces an immediate LH pulse, it also resets the GnRH pulse generator, resulting in a delay in the appearance of the subsequent endogenous pulse. We now explore the effects of kisspeptin on the GnRH pulse generator in women.

Methods: Eight women in the follicular phase (d 2 to 5 of the menstrual cycle) and 16 women in the mid-luteal phase (d -9 to -5) were admitted to the MGH Harvard Catalyst Clinical Research Center (HCCRC) for 12 h of q10 min blood sampling to chart LH pulses. Kisspeptin 112-121 0.24 nmol/kg (0.31 mcg/kg) was given intravenously at the 6h time point.

Results: Kisspeptin elicited an immediate LH pulse in all women in the mid-luteal phase. In contrast, only 4 of 8 women in the follicular phase exhibited a rise in LH after kisspeptin administration. Although in men, the mean amplitude of kisspeptin-induced LH pulses is larger than that of endogenous pulses, in luteal-phase women, the kisspeptin-induced LH pulses and endogenous pulses had comparable amplitudes (3.9 ± 0.6 vs. 3.4 ± 0.4 mIU/mL, $p = 0.8$). In men, kisspeptin [ldquo]resets[rdquo] the GnRH pulse generator, but in luteal phase women, there was no evidence of resetting of the pulse generator, as the interval between the endogenous pulses immediately preceding and following the kisspeptin-induced pulse (239 ± 38 min) was no different from the pulse interval in healthy cycling women in the mid-luteal phase (202 ± 24 min, $p = 0.5$).

Conclusions: There are marked differences in the responses of follicular-phase women, luteal-phase women, and men to the same dose of kisspeptin. Kisspeptin stimulates LH pulses in both men and luteal-phase women, but not consistently in follicular-phase women. Kisspeptin resets the GnRH pulse generator in men but not in women. These observations may represent different sensitivities of the GnRH secretory apparatus to kisspeptin dose, or may reflect fundamental differences in the response to kisspeptin. Future work will determine whether these differences are due to differences in sex-steroid environments or to other factors.

Nothing to Disclose: Y-MC, JPB, VFS, NEP, JEH, SBS

Pub # P3-151

Session Information POSTER SESSION: BASIC - Reproductive Neuroendocrinology (1:30 PM-3:30 PM)

Title Stereologic Analysis of Progesterone Receptor in the Hypothalamus of Female Rhesus Macaques and Its Regulation by Aging and Estrogen

Author String MM Naugle, LT Nguyen, TK Merceron, AC Gore
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Body The progesterone receptor (PR) in the hypothalamus is involved in the neuroendocrine control of reproduction. Progesterone (P), acting on the PR, works in concert with estrogen to mediate negative feedback of sex steroids, and in females, to enhance the preovulatory GnRH/LH surge, which triggers ovulation. The progesterone receptor (PR) is heavily expressed in many of the neurons that modulate GnRH, the peptide that is known as the master control of the reproductive system in vertebrates, although not within GnRH neurons themselves. Several studies have investigated changes in PR expression and regulation by hormones and aging in rats. Those results showed that PR expression decreases with age, and that estradiol treatment up-regulates its expression in the rodent hypothalamus. Relatively little is known about the regulation of PR in the primate brain, though it has been shown that the capacity to respond to P erodes during the process of aging. Our hypothesis is that the age-related decrease in P sensitivity is due to a decrease in the number of cells that express PR in the hypothalamus. In the present study, the hypothalami of ovariectomized (OVX) female rhesus macaque monkeys were used at two ages: young adult (~12 years) and aged adult (~21 years). These animals received injections of either peanut oil (vehicle) or estradiol cypionate every 27 days for 2 years. Monkeys were humanely euthanized and transcardially perfused with 4% paraformaldehyde in PBS. The hypothalamus was sectioned at 50um with a vibratome and serial sections were collected. We used immunocytochemistry, brightfield microscopy and stereology to quantify the number of PR-expressing cells in hypothalamic nuclei that provide modulatory input to GnRH neurons: the arcuate (ARC) and the anteroventral periventricular area (AVPe). In both of the nuclei analyzed, no effect of estradiol on the number of PR-cells was found. In the AVPe, but not the ARC, an age effect was observed, with the expression of PR in aged animals significantly higher than in the young adult. These results indicate that the age-related decrease in progesterone sensitivity is not related to a change in the number of cells that express PR in the ARC or AVPe in the rhesus macaque. These results are surprising because they differ from what is found in rodent models and highlight the importance of using primate models to investigate mechanisms underlying problems in human health.

Sources of Research Support: NIH Grant support: NIH AG16765.

Nothing to Disclose: MMN, LTN, TKM, ACG

Pub # P3-152

Session Information POSTER SESSION: BASIC - Reproductive Neuroendocrinology (1:30 PM-3:30 PM)

Title Ultrastructural Properties of the Median Eminence Are Altered during Reproductive Aging in the Female Rhesus Macaque

Author String MM Naugle, FA Guarraci, W Yin, AC Gore
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Body Gonadotropin-releasing hormone (GnRH) is the driving force for the hypothalamic-pituitary-gonadal (HPG) axis, which controls reproductive function. The GnRH decapeptide is released from neuroterminals in the median eminence (ME), where synapses are sparse and cellular signaling is dominated by volume transmission. Dramatic organizational changes in the GnRH-glial-capillary network in the ME have been shown using an aging rodent model. In both rodents and primates, aging is associated with an erosion of the capacity of the brain to respond to sex steroid hormones, such as estradiol (E2). Here, using an aging non-human primate model we examined effects of age and hormone treatment on the anatomical changes in the ME. We hypothesized that the dysregulation of GnRH by sex steroid hormones observed in aged animals is due, in part, to anatomical changes that occur in the ME. We used the hypothalami of ovariectomized (OVX) female rhesus macaque monkeys at two ages: young (~12 years) and aged (~21 years) adults. These animals received injections of peanut oil (vehicle), estradiol cypionate (E2), or E2 + progesterone (P4) every 28 days for 2 years. The monkeys were humanely euthanized and transcardially perfused with 4% paraformaldehyde in PBS. The ME was blocked and embedded in lowicryl resin. Ultrathin sections were collected using an ultramicrotome and imaged using a transmission electron microscope (TEM). Preliminary data showed that the ME of aged primates underwent structural disorganization. The number of neuroterminals decreased while their total volume increased. The basal lamina became convoluted and glial cells changed shape from linear to globular. These results suggest that deterioration of the ME organization may affect the release of GnRH and other releasing hormones synthesized in the hypothalamus with wide-ranging physiological impacts that may contribute to the aging process.

Sources of Research Support: NIH Grant support: NIH AG16765.

Nothing to Disclose: MMN, FAG, WY, ACG

Pub # P3-153

Session Information POSTER SESSION: BASIC - Reproductive Neuroendocrinology (1:30 PM-3:30 PM)

Title Sensitivities of mRNA Expression Levels of Kiss1 and Its Receptor, Kiss1r, to Nutritional Status Are Changed during the Developmental Period in Female Rats

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Body

OBJECTIVES
Decreased activity of kisspeptin, the product of the hypothalamic Kiss1 gene, is the major cause of the suppression of reproductive function in subnutritional conditions. We examined the developmental changes in the sensitivity of hypothalamic mRNA expression of Kiss1 and its receptor, Kiss1r to nutritional status in female rats.

METHODS
Female Sprague-Dawley rats (Charles River Japan, Inc., Tokyo, Japan) were used. Effects of food deprivation (12h or 24h) on the plasma leptin and LH concentrations and the hypothalamic Kiss1, Kiss1r, and GnRH mRNA levels were evaluated in postnatal day 5, 15 and 25 rats. In addition the effects of the co-administration of leptin on 24h food deprivation-induced alterations in hypothalamic neuropeptide mRNA levels were evaluated in postnatal day 25 rats.

RESULTS
Kiss1 mRNA expression was reduced by 24 h food deprivation at day 25, but not at day 5 or day 15 rats. Kiss1r mRNA expression was reduced by 12h or 24h food deprivation at postnatal days 5 and 25, but not at postnatal day 15. Kiss1r mRNA level was found to be correlated with the plasma leptin level, and the administration of leptin restored the 24h food deprivation-induced suppression of Kiss1r mRNA expression in postnatal day 25 rats.

CONCLUSION
Hypothalamic Kiss1 and Kiss1r mRNA expression is differentially affected by nutritional condition at different age-points. The sensitivity of Kiss1 mRNA to nutritional status might develop during the neonatal period. On the other hand, the sensitivity of Kiss1r mRNA to nutritional status has been already established during the early neonatal period. Hypoleptinemia plays a role in the reduction of hypothalamic Kiss1r mRNA expression under subnutritional conditions.

Nothing to Disclose: TM, TI, RK, GG, MI

Pub # P3-154

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Uterus, Placenta & Pregnancy, Including Endometriosis (1:30 PM-3:30 PM)

Title The miR-200 Family Enhances Contractility of the Pregnant Uterus near Term Via Downregulation of STAT and Induction of 20 α -Hydroxysteroid Dehydrogenase (20 α -HSD)

Author String NE Renthal, KC Williams, CR Mendelson
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Body Throughout most of pregnancy, uterine quiescence is maintained by increased progesterone receptor (PR) transcriptional activity, while spontaneous labor is facilitated by a concerted series of biochemical events that activate inflammatory pathways and impair PR function. Recently, we uncovered a new regulatory pathway whereby microRNAs (miRNAs, miRs) serve as hormonally-modulated mediators of contraction-associated genes in pregnant uterus from mouse to human (1). We identified a conserved miRNA family, the miR-200 family, that is highly induced at term in myometrium of both mice and humans, as well as two coordinately downregulated targets, zinc finger E-box binding homeobox proteins, ZEB1 and ZEB2, which act as transcriptional repressors. We further demonstrated that ZEB1 is directly upregulated by the action of P4/PR at the ZEB1 promoter. Importantly, ZEB1 and ZEB2 inhibit expression of the contraction-associated genes, oxytocin receptor and connexin-43 and block oxytocin-induced myometrial contractility (1). It has been postulated that PR function near term is compromised in myometrium by decreased expression of PR coactivators, altered expression of PR isoforms and increased local metabolism of P4 to inactive products. Previously, we observed that activity of the P4-metabolizing enzyme, 20 α -HSD, was increased dramatically in the pregnant mouse uterus near term and was upregulated by labor-inducing signals (2). Interestingly, one of the predicted targets of miR-200 family is the transcription factor, signal transducer and activator of transcription (STAT)5b, which is known to inhibit 20 α -HSD gene expression (3). In the present study, we tested the hypothesis that the miR-200 family serves as an important regulator of myometrial P4 metabolism near term via inhibition of STAT-5b and upregulation of 20 α -HSD. Using RT-qPCR and immunoblotting, we observed that 20 α -HSD (AKR1c18) mRNA and protein increased markedly in pregnant mouse myometrium between 15.5 days post-coitum (dpc) and labor (19.0 dpc). This was associated with a coordinate decline of STAT5b mRNA. Interestingly, we observed that overexpression of miR-200 family in cultured human myometrial cells markedly suppressed STAT5b mRNA levels. Together, these findings implicate the miR-200 family as an important regulator of increased local P4 metabolism in the pregnant uterus near term and may provide insight into its importance in the decline in PR function leading to labor.

(1) Renthal NE, Chen CC, Williams KC, Gerard RD, Prange-Kiel J, Mendelson CR 2010 miR-200 family and targets, ZEB1 and ZEB2, modulate uterine quiescence and contractility during pregnancy and labor. *Proc Nat Acad Sci U S A* 107:20828-20833

(2) Condon JC, Mendelson CR 2004 Fetal macrophages, which contain elevated 20 α -hydroxysteroid dehydrogenase (20 α -HSD) activity and invade the pregnant uterus near term, may contribute to the onset of labor by causing local progesterone withdrawal. *J Soc Gynecol Invest* 11:224A

(3) Piekorz RP, Gingras S, Hoffmeyer A, Ihle JN, Weinstein Y 2005 Regulation of progesterone levels during pregnancy and parturition by signal transducer and activator of transcription 5 and 20 α -hydroxysteroid dehydrogenase. *Mol Endocrinol* 19:431-440

Sources of Research Support: NIH P01-HD011149; March of Dimes Birth Defects Foundation Prematurity Initiative Grant 21-FY07-601.

Nothing to Disclose: NER, KCW, CRM

Pub # P3-155

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Uterus, Placenta & Pregnancy, Including Endometriosis (1:30 PM-3:30 PM)

Title Decreased Expression of the miR-199a/214 Cluster Mediates Enhanced Cyclooxygenase-2 (COX-2) Expression and Contractility in Mouse Myometrium during Term and Preterm Labor

Author String KC Williams, NE Renthall, CR Mendelson
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Body In studies using miRNA array analysis of myometrial tissues from pregnant mice at 15.5 vs. 18.5 days post-coitum (dpc) (term = 19.0 dpc), we identified a conserved cluster of miRNAs, miR-199a/214 cluster, that is downregulated in mouse myometrial tissues near term. The miR199a/214 cluster includes miR-199a-3p, miR-199a-5p and miR-214, and is synthesized as part of a 6-kb anti-sense transcript (Dnm3os), which is highly expressed in pregnant uterus (1). Interestingly, miR-199a-3p and miR-214 are known and predicted to target cyclooxygenase 2 (COX-2) mRNA, respectively (2,3). COX-2 is a key regulatory enzyme in the synthesis of prostaglandins, which are potent stimulators of uterine contractility. RT-qPCR of RNA isolated from myometrial tissues of pregnant mice between 15.5 and 18.5 dpc confirmed that both miR-199a-3p and miR-214 were downregulated toward term and significantly decreased during labor. Notably, expression of COX-2 mRNA remained low in the pregnant myometrium until active labor when its expression was markedly induced. Luciferase reporter assays in COS7 cells cotransfected with *COX2-3'-UTR-luciferase* reporters revealed that miR-214-induced downregulation of COX-2 expression was caused by direct binding of miR-214 to COX-2 3'-UTR. In cultured human myometrial cells, overexpression of miR-199a-3p and miR-214 caused suppression of COX-2 mRNA and protein, confirming their inhibitory role in myometrium. To assess the regulation of miR-199a-3p/214 expression in preterm labor, 15.5 dpc pregnant mice were injected intraamniotically with lipopolysaccharide (LPS), which induced preterm labor within 12-16 h. Interestingly, LPS treatment caused a significant inhibition of miR-199a-3p and miR-214 expression and an associated induction of COX-2 mRNA in the preterm laboring mouse myometrium, suggesting a role of these miRNAs in inflammatory preterm labor. Based on these intriguing findings, we postulate that elevated expression of myometrial *miR-199a/214* throughout most of pregnancy maintains uterine quiescence by inhibiting expression of COX-2, thereby blocking the synthesis of prostaglandins. At term, an increased inflammatory response causes the downregulation of *miR-199a/214* expression with an associated increased in COX-2, resulting in a further upregulation of the inflammatory response and expulsion of the fetus. Studies are in progress to identify the hormones and factors that regulate *miR-199a/214* expression during pregnancy and labor.

- (1) Watanabe et al., Dev Dyn 2008; 237:3738
- (2) Chakrabarty et al., Proc Natl Acad Sci USA 2007;104:15144
- (3) Grimson et al., Mol Cell 2007; 27:91

Sources of Research Support: NIH P01-HD011149; March of Dimes Birth Defects Foundation Grant 21-FY07-601.

Nothing to Disclose: KCW, NER, CRM

Pub # P3-156

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Uterus, Placenta & Pregnancy, Including Endometriosis (1:30 PM-3:30 PM)

Title Functional Role of the MicroRNA (miR)-17-92 Cluster during Human Trophoblast Differentiation

Author String P Kumar, CR Mendelson
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Body To identify miRNAs involved in the regulation of human trophoblast differentiation and the associated induction of aromatase/*hCYP19* and human chorionic gonadotropin β (*hCG\beta*) expression, we performed miRNA microarray analysis of RNA from mononuclear cytotrophoblasts (CYT) isolated from midgestation human placenta before (0 h) and after 24 and 48 h of culture. During this period, CYT spontaneously fuse to form syncytiotrophoblast (SYN) with concomitant induction in *hCYP19* and *hCG\beta* expression. Analyses of the microarray data revealed 16 miRs that were significantly ($p < 0.05$) downregulated and 7 miRs that were upregulated >2 -fold at both 24 and 48 h, as compared to 0 h. Downregulated miRNAs included several members of the polycistronic miR-17-92 cluster (miR-17, 19b, 20a). Using TargetScan analysis, we identified several predicted mRNA targets of these miRs that are of potential importance in trophoblast differentiation. These included CYP19 mRNA itself, and GCM1, a transcription factor critical for labyrinthine trophoblast development and CYP19 expression. Intriguingly, these miRs also are known to target estrogen receptor α , which is upregulated during trophoblast differentiation and essential for induction of *CYP19* expression. Downregulated expression of several members of miR-17-92 cluster during syncytiotrophoblast differentiation was confirmed using quantitative real-time SYBR[reg] Green- and TaqMan-based RT-qPCR. Importantly, overexpression of miR-19b, a member of the miR-17-92 cluster in cultured human trophoblast cells caused a significant decrease in *hCYP19* mRNA and protein expression, suggesting *hCYP19* mRNA as a target of miR-19b. Overexpression of miR-19b also inhibited expression of GCM1 protein and *hCG\beta* mRNA in cultured human trophoblasts. Luciferase reporter assays in JEG3 cells cotransfected with *hCYP19*- and GCM1-3'UTR-luciferase reporters revealed that miR-19b-induced downregulation of *hCYP19* and GCM1 expression was caused by direct binding of miR-19b to predicted sequences in their 3'UTRs. Mutagenesis of the putative miRNA binding sites are in progress to confirm these interactions. Since several studies have shown that overexpression of miR-17-92 cluster promotes proliferation, suppresses apoptosis and induces angiogenesis in other cell types, studies also are in progress to elucidate the roles of the miR-17-92 cluster in human cytotrophoblast proliferation and in negative regulation of key SYN genes involved in trophoblast differentiation.

Sources of Research Support: NIH R01 DK031206.

Nothing to Disclose: PK, CRM

Pub # P3-157

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Uterus, Placenta & Pregnancy, Including Endometriosis (1:30 PM-3:30 PM)

Title *Plac1* (Placenta-Specific 1) Is Necessary for Normal Placental and Embryonic Development

Author String SM Jackman, ME Fant, X Kong
University of South Florida College of Medicine, Tampa, FL; University of South Florida College of Medicine, Tampa, FL

Body **Introduction:** *Plac1* is a recently identified gene located at Xq26. Its expression is highly restricted to cells of trophoblast lineage and is membrane-associated. Although it localizes to a region thought to be important in placental growth its function is unknown. The aim of this study was to determine if *Plac1* is necessary for normal placental and embryonic development in a mouse knockout model.

Methods: The open reading frame of the *Plac1* gene was deleted in murine ES cells and bred against a C57BL/6 background. Timed matings were established between wild type (WT) males and heterozygous females (hets). Pregnant females were sacrificed at days 16.5 and 18.5 gestation. The placentas and embryos were weighed and immediately placed in 4% paraformaldehyde for further processing. Placental structure was analyzed using standard immunohistochemical (IHC) techniques.

Results: *Plac1* deletion resulted in significant placentomegaly and intrauterine growth retardation (IUGR). At d16.5 of gestation heterozygous placentas derived from WT male X female het matings weighed 100% (50-150%) more than WT placentas, whereas the corresponding embryos weighed 12% (7-19%) less than their WT littermates. By d18.5, female het (+/-mat) placentas weighed 88% (72-104%) more than the WT placentas, and the corresponding embryos weighed 6.5% (0-13%) less than their WT littermates. IHC staining revealed major structural abnormalities in the affected placentas including a markedly expanded spongiotrophoblast that invaded a disorganized labyrinth and excessive glycogen cells within the spongiotrophoblast layer. Because the paternal X-chromosome is preferentially inactivated in extraembryonic tissue, the heterozygous (+/-mat) genotype likely represents the null phenotype. While male embryos hemizygous for the *Plac1* null-allele are potentially viable and fertile they appear to be underrepresented in the litters described above and among the surviving offspring. These preliminary observations suggest that *Plac1* deletion may also variably influence fetal viability in a gender-dependent manner. Further analyses are ongoing to confirm this effect.

Conclusion: *Plac1* is essential for normal placental development and function. Its disruption results in IUGR, demonstrating it is also necessary for normal fetal development. Studies are ongoing to further characterize the impact of *Plac1* deletion on placental and embryonic development and to determine its mechanism of action at the cellular level.

Sources of Research Support: March of Dimes #6-FY09-503.

Nothing to Disclose: SMJ, MEF, XK

Pub # P3-158

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Uterus, Placenta & Pregnancy, Including Endometriosis (1:30 PM-3:30 PM)

Title The Timing of Parturition Is Regulated by Myometrial Endoplasmic Reticulum Stress and the Unfolded Protein Response

Author String A Suresh, K Subedi, P Jeyasuria, J Condon
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Body During the final trimester of pregnancy, the uterus transitions from a hyperplastic to a hypertrophic state and uterine myocytes must stretch to accommodate the rapidly growing fetus as term approaches. We previously determined that uterine caspase-3 functions in a non-apoptotic manner to maintain uterine quiescence during the final trimester of pregnancy by disabling both structural and non-structural components of the uterine myocyte contractile apparatus. As term approaches, caspase-3 declines to barely detectable levels permitting reconstitution of the uterine myocyte contractile architecture and an increased contractile potential(1). In this current study, we have isolated a novel and likely physiologically relevant signaling cascade that may act as a master regulator of uterine quiescence and contractility by regulating uterine caspase-3 activation and its repression during late gestation. Our work demonstrates that uterine caspase-3 is activated by elevated levels of endoplasmic reticulum stress (ERS) that occurs in response to hypoxic and mechanical challenges during the transition of the pregnant uterus into its hypertrophic state. Activation of ERS and its resolution by an unfolded protein response (UPR) is a mechanism utilized by cells to withstand environmental stressors. We suggest that the pregnant uterine myocyte partners with the UPR to limit the tocolytic action of uterine caspase-3 as term approaches by hosting a pro-survival anti-apoptotic response. Utilizing pregnant mouse uteri, we extracted RNA and isolated cytoplasmic, nuclear and microsomal protein fractions from gestational day (E) 10-19. We identified an upregulation of the caspase activation arm of the UPR from E12-14. Increased levels of PERK, eIF2 α and CHOP along with caspase-12, -9 and -3 activation were found from E12-14. A marker of the pro-adaptive UPR, BiP, which functions to restore endoplasmic reticulum homeostasis, was markedly elevated on E13-15. An anti-apoptotic, pro-survival surge that accommodates the restorative arm of the UPR was also activated as evidenced by upregulation of NF-[kappa] B and members of the IAP family such as Mcl-1 from E14 to term. Thus, we propose that uterine quiescence is maintained by an ERS induced caspase-3 activation. Consequently a downstream pro-survival UPR is triggered that acts to limit active caspase-3. The precise timing of these events functions to secure an appropriate gestational length thereby avoiding the onset of pre-term birth.

(1) Jeyasuria P et al., Biol Reprod 2009;80(5):928-34

Sources of Research Support: March of Dimes 21-FY2010-555.

Nothing to Disclose: AS, KS, PJ, JC

Pub # P3-159

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Uterus, Placenta & Pregnancy, Including Endometriosis (1:30 PM-3:30 PM)

Title Differential Expression of Kisspeptin and Neurokinin B in Trophoblast Subpopulations of Human Placenta

Author String R Upadhyay, C Fortique, JC Gill, CP Crum, RS Carroll, UB Kaiser, W Kuohung
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Body Prior to the discovery of its role in reproduction, kisspeptin was initially found to inhibit metastasis in melanoma and breast cancer. Kisspeptin is highly expressed in the placenta, a major source of circulating kisspeptin in the human. Encoded by the gene KISS1, kisspeptin is hypothesized to regulate trophoblast invasion required for maternal vascular remodeling and sufficient blood flow to the developing fetus. Recently, KISS1 neurons in the arcuate nucleus of the hypothalamus were found to co-express neurokinin B (NKB), and an interdependent role for the two neuropeptides in the sex steroid feedback regulation of hypothalamic GnRH release has been suggested. Similarly, NKB has also been reported to be highly expressed in the human placenta. The roles of these neuropeptides in pregnancy and whether they are linked as in the hypothalamus are still unclear. Further characterization of their expression profiles within the heterogeneous placenta is needed. In this study, we performed enzymatic digestion and density gradient fractionation of primary human placental cells to isolate syncytiotrophoblasts, the multinucleated cell layer where maternal-fetal nutrient exchange occurs, and cytotrophoblasts, the invasive cells of the placenta. Relative expression levels of KISS1 and TAC3 (gene encoding NKB) in the two enriched fractions were measured by RT-qPCR. Both KISS1 and TAC3 mRNA levels in 11-12 week and 16-17 week gestational age placentas were significantly higher in the syncytiotrophoblast fraction than in the cytotrophoblast fraction. This KISS1 expression pattern was confirmed by immunofluorescent co-staining of placental sections of similar gestational age with polyclonal antibodies to kisspeptin and hepatocyte growth factor activator inhibitor type 1 (HAI-1), a villous cytotrophoblast-specific marker. The staining was mutually exclusive, with kisspeptin localizing in the cytoplasm of the outer syncytiotrophoblast layer and HAI-1 in the inner villous cytotrophoblast layer. Localization of NKB and kisspeptin to the syncytiotrophoblast compartment supports previous hypotheses that a paracrine mechanism may be involved in regulating invasive cytotrophoblasts. Further characterization of the expression of these neuropeptides and their receptors within the placenta may help to elucidate the mechanisms underlying disorders of poor placental invasion such as preeclampsia.

Nothing to Disclose: RU, CF, JCG, CPC, RSC, UBK, WK

Pub # P3-160

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Uterus, Placenta & Pregnancy, Including Endometriosis (1:30 PM-3:30 PM)

Title PPAR α Inhibits, Whereas PPAR γ Stimulates, the Expression of 11 β -Hydroxysteroid Dehydrogenase Type 2 in Human Placenta

Author String X Ni, P He, Y Li, H Gu
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Body The placental 11 β -hydroxysteroid dehydrogenase type 2 (11 β -HSD2) serves a functional barrier to protect the fetus from excessive exposure to high levels of maternal glucocorticoid by converting maternal cortisol to cortisone. Peroxisome proliferator-activated receptors (PPARs) have been implicated to play a pivotal role in placental function and fetal development, and PPAR[delta] has been shown to suppress 11 β -HSD2 expression in human placental cells. However, the roles of PPAR and PPAR γ in the control of 11 β -HSD2 expression remain unknown. The purpose of the present study was to investigate the modulation of 11 β -HSD2 by PPARs in cultured placental trophoblasts. Treatment of placental cells with PPAR α agonist GW7647 caused a decrease in the mRNA and protein expression as well as the activity of 11 β -HSD2. These effects could be blocked by PPAR α antagonist GW7647. PPAR γ agonist rosiglitazone dose-dependently stimulated the expression and activity of 11 β -HSD2. PPAR γ antagonist GW9662 reversed the effects of rosiglitazone. RXR ligand 9-cis-RA potentiated the effects induced by PPARs agonists. Cells were treated with PPARs agonists in the absence and presence of CHX, a protein synthesis inhibitor. It was found that GW7647 and rosiglitazone were equally effective in modulating 11 β -HSD2 mRNA in the absence and presence of CHX, indicating that de novo protein synthesis was not required. GW7647 and rosiglitazone did not alter the half-life of 11 β -HSD2 mRNA. GW7647 repressed whereas rosiglitazone stimulated the promoter activity of 11 β -HSD2 gene. Taken together, these data suggest that PPAR α and PPAR γ differentially regulates the expression and activity of 11 β -HSD2 in human trophoblast cells, these effects are mediated primarily at transcriptional level. Thus, the present study indicate that PPARs may modulate the human placental function. and, consequently, fetal development.

Sources of Research Support: Natural Science Foundation of China 30800391&31071314; Science and Technology Commission of Shanghai Municipals 09XD1405600.

Nothing to Disclose: XN, PH, YL, HG

Pub # P3-161

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Uterus, Placenta & Pregnancy, Including Endometriosis (1:30 PM-3:30 PM)

Title Endometrial Angiogenic Effects of Human Chorionic Gonadotropin Appear To Be Independent of Vascular Endothelial Growth Factor

Author String SM Thompson, A Wojtczuk, D Cole, G Weiss, LT Goldsmith
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Body Endometrial angiogenesis is required for normal menstrual cyclicity and establishment of pregnancy. The specific mediators are poorly defined. Although human chorionic gonadotropin (hCG) fosters endometrial angiogenesis, the mechanisms involved are unclear. Few studies have attempted to define the role of VEGF in hCG-mediated endometrial angiogenesis. To help clarify the mechanisms by which hCG regulates angiogenesis, the current studies tested the hypothesis that hCG stimulates expression of VEGF in a well established in vitro model of human endometrial function, telomerase-immortalized human endometrial stromal cells. After serum starvation, cells were incubated in the absence (control) or presence of hCG, at 10 or 100 IU/ml for 48 and 72 hours (h). As a stimulatory positive control, cells were incubated with 8-bromo-cAMP. Since hCG stimulates endometrial stromal Interleukin-11 (IL-11), a cytokine essential for implantation, hCG-stimulated IL-11 was also used as a positive control. VEGF and IL-11 levels in conditioned media from each replicate well were measured using specific ELISAs and are expressed as mean±SEM, n=4 experiments each conducted in triplicate. Differences between groups were assessed using ANOVA and Mann-Whitney tests. hCG significantly increased IL-11 expression, as we have shown previously. IL-11 levels were increased from 81±9 pg/ml (control, untreated cells) to 257±57 by 10 IU hCG (p<0.0001) and to 466±57 by 100 IU hCG (p<0.0001) at 48h and from 103±21 pg/ml (control cells) to 197±20 by 10 IU hCG (p=0.003) and to 337±31 by 100 IU hCG (p<0.0001) at 72h. In contrast, VEGF levels were not significantly stimulated by either dose of hCG at either time point (p>0.05 for 10 or 100 IU at 48 and 72h). Media VEGF levels from control, untreated cells were 91±8 pg/ml and 58±5 and 72±10 from cells incubated with 10 and 100 IU hCG respectively at 48h, and at 72h were 135±17 pg/ml in media from control cells and 106±14 and 63±8 from cells incubated with 10 and 100 IU hCG respectively; whereas incubation with 0.5 mM 8-bromo-cAMP significantly increased media VEGF levels from 91±8 to 351±40 at 48h (p<0.001) and from 135±17 to 620±76 at 72h (p<0.001). These data demonstrate that, in contrast to the effect of other hormones and local factors such as estradiol, progesterone and relaxin, hCG does not directly regulate endometrial VEGF production. Other mechanisms are therefore necessary to mediate the angiogenic effects of hCG in the endometrium.

Nothing to Disclose: SMT, AW, DC, GW, LTG

Pub # P3-162

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Uterus, Placenta & Pregnancy, Including Endometriosis (1:30 PM-3:30 PM)

Title Novel Roles of Corticotropin-Releasing Hormone in the Expression of Regulators of Cellular Sensitivity to Glucocorticoid Actions in BeWo Trophoblast Cells

Author String AC Hein, M Delidaki, M Gu, M Vatish, D Grammatopoulos
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Body During human pregnancy, the placenta expresses complex machinery (the [ldquo]glucocorticoid barrier [rdquo]) to dynamically control (and limit) fetal exposure to glucocorticoids (GC). To understand how the placental [ldquo]GC barrier[rdquo] is regulated, we employed the BeWo choriocarcinoma cell line, able to differentiate upon forskolin treatment. Morphological and biochemical differentiation of these cells was confirmed by E-cadherin immunostaining and immunofluorescent confocal microscopy, and measurements of hCG secretion. Using real-time RT-PCR, we identified expression of mRNA transcripts for 11beta-hydroxysteroid dehydrogenase type 2 (11beta-HSD2), the enzyme which converts active GCs to their inactive derivatives, and the multidrug resistance transporter P-glycoprotein (P-gp), which is potentially capable of protecting the fetus from a large number of drugs and toxins, as well as exporting GCs out of the cells. Forskolin-induced BeWo cell differentiation was associated with a significant time-dependent increase in both 11beta-HSD2 and P-gp mRNA expression levels by 5- and 4-fold respectively. Protein levels of 11beta-HSD2 were also substantially elevated in differentiated BeWo cells. Incubation of undifferentiated or differentiated BeWo cells with dexamethasone did not alter 11beta-HSD2 or P-gp mRNA, suggesting that the expression of the molecular components of the placental [ldquo]GC-barrier[rdquo] is not directly regulated by GC. This is in contrast to the mouse placenta where dexamethasone exposure up-regulates P-gp mRNA. Forskolin also significantly upregulated mRNA of type 1 receptor for corticotropin-releasing hormone (CRH-R1) expression a signaling event that might increase cellular responsiveness of differentiated BeWo trophoblasts to CRH actions. Indeed, in differentiated, but not undifferentiated, BeWo cells CRH was able to significantly enhance 11beta-HSD2 and P-gp mRNA expression levels. The CRH-R1 expressed in the differentiated BeWo trophoblasts activates a complex network of signaling molecules including ERK1/2 and p38MAPK, PI3K and Akt, molecules implicated in the regulation of P-gp expression. Thus, this data identified a novel aspect of placental actions of CRH in the BeWo differentiated trophoblast involving regulation of molecules that regulate tissue sensitivity to GC with potentially important pathophysiological implications.

Sources of Research Support: Wellcome Trust/NIH studentship awarded to ACH.

Nothing to Disclose: ACH, MD, MG, MV, DG

Pub # P3-163

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Uterus, Placenta & Pregnancy, Including Endometriosis (1:30 PM-3:30 PM)

Title Transcription Factor Interactions in the Regulation of Human Uterine Stromal Cell Differentiation

Author String S Handwerger, JK Schroeder, CA Kessler
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Body Previous studies from our laboratory demonstrated that the transcription factors ETS1, FOXO1 and TWIST1 are critical for the induction of human uterine stromal cell differentiation (decidualization). Knockdowns of each of the three transcription factors markedly attenuated the morphologic differentiation of human uterine fibroblast (HUF) cells in response to progesterone, estradiol and PGE2 as well as the induction of decidualization-specific genes such as PTHrP, lefty2 and prolactin. PTHrP, lefty2, and prolactin in turn acted in an autocrine manner to inhibit further decidualization of the stromal cells, thereby limiting the extent of the differentiation process. In the present study, we demonstrated that the inhibition of decidualization by PTHrP, lefty2, and prolactin resulted in marked inhibition of ETS1, FOXO1 and TWIST1, further supporting pivotal roles for the three transcription factors in the regulation of decidualization. Additional studies demonstrated interactions among the three transcription factors. Silencing the expression of ETS1 (by a morpholino oligomer), FOXO1 or TWIST1 (both by siRNAs) prior to the induction of in vitro decidualization by progesterone, estradiol and PGE2 repressed mRNA and protein levels of the other two transcription factors by 60 to 70 percent. Computer analyses and transient transfection studies in HUF cells of the proximal promoters of the three transcription factors provided additional evidence supporting a feedback loop between the transcription factors. For example, the FOXO1 promoter has four ETS sites, the TWIST1 promoter has three ETS sites and overexpression of ETS1 transactivates the ETS1 and FOXO1 promoters in HUF cells undergoing decidualization. Taken together, these investigations strongly suggest that ETS1, FOXO1 and TWIST1 form a novel network of transcription factors that interact to regulate human uterine stromal cell differentiation. Upregulation of the transcription factors is critical for the induction decidualization. Autocrine factors that subsequently act to limit decidualization downregulate the transcription factors.

Sources of Research Support: NIH Grant HD05201(SH).

Nothing to Disclose: SH, JKS, CAK

Pub # P3-164

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Uterus, Placenta & Pregnancy, Including Endometriosis (1:30 PM-3:30 PM)

Title The BMP Receptors ALK2 and ALK3 Are Essential for Fertility of Female Mice

Author String C Clementi, MA Edson, MJ Large, R Chen, VM Kaartinen, JP Lydon, FJ DeMayo, MM Matzuk
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Body To achieve a healthy pregnancy, the embryo and the uterus must establish a cross talk that will be essential throughout gestation. The implantation of the embryo takes place in a limited window of time when the uterus is receptive and the embryo has reached the proper stage of development. This crucial synchrony is controlled by the sex hormones through the regulation of the expression of many downstream factors; intriguingly, these factors are connected by mutual regulation and feedback loops that make this regulatory network challenging to disentangle.

BMP2 is one of these factors. Mice lacking this TGF β superfamily member in the uterus cannot undergo the morphological and functional transformation of the endometrium that normally follows the attachment of the embryo(1). This transformation, called decidualization, is a fundamental step in the implantation process and is essential for preparing the uterus for the sheltering of the embryo. BMP2 signals through heterodimers of BMP type 1 and type 2 serine/threonine kinase receptors. A BMP ligand can bind different receptors in different contexts: the physiological association with a specific receptor depends on both the binding affinity and the actual availability of the ligand and the receptor in a specific environment. While the type 2 receptors are essential for recognizing the ligands, the type 1 receptors determine the specificity of the intracellular response. Three type 1 receptors, ALK2, ALK3, and ALK6, are known to mediate BMP signaling, and all expressed in the pregnant uterus. To study the implantation process, we investigated the effects of knocking out each of these receptors in the uterus. Although ALK6 null mice are sterile, this receptor is not required for the decidualization(2). We now demonstrate that ALK2 and ALK3 are essential for the mouse fertility and are required during the peri-implantation period. Moreover, the differences presented by the ALK2 and ALK3 cKO mice suggest that the involvement of the TGF β signaling in the regulation of the process goes beyond what is known for BMP2 and affects the early pregnancy at different steps. In particular, we discovered that ALK3 plays a role in the pre-implantation period, whereas absence of ALK2 affects the system at a later stage.

Considering the broad involvement of the TGF β superfamily in many processes, it is crucial to further dissect these pathways in uterine biology to identify possible targets for future therapeutic manipulation.

(1) Lee KY, Jeong JW, Wang J, Ma L, Martin JF, Tsai SY, Lydon JP, DeMayo FJ. Mol Cell Biol. 2007 Aug;27(15):5468-78. Epub 2007 May 21.

(2) Edson MA, Nalam RL, Clementi C, Franco HL, Demayo FJ, Lyons KM, Pangas SA, Matzuk MM. Mol Endocrinol. 2010 Jun;24(6):1251-66. Epub 2010 Apr 2.

Sources of Research Support: NIH Grant HD32067.

Nothing to Disclose: CC, MAE, MJL, RC, VMK, JPL, FJD, MMM

Pub # P3-165

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Uterus, Placenta & Pregnancy, Including Endometriosis (1:30 PM-3:30 PM)

Title Serum Umbilical Cord VEGF and sFlt-1 Levels in Preeclampsia: Effects of IGF-I upon the Secretion of These Two Angiogenic Factors by HUVEC

Author String A Olmos, L Diaz, D Barrera, E Avila, F Larrea, A Halhali
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Body **Introduction and objective:** Since insulin-like growth factor-I (IGF-I) stimulates angiogenesis and its concentrations are decreased in preeclampsia (PE), disease associated with decreased angiogenic process, the aim of the present study was to determine serum umbilical cord levels of vascular endothelial growth factor (VEGF) and the soluble form of its receptor 1 (sFlt-1), and to assess the effects of IGF-I upon the secretion of these two angiogenic factors by cultured human umbilical vein endothelial cells (HUVEC) in PE. **Materials and methods:** Serum umbilical cord samples were obtained from 9 PE and 18 normotensive (NT) pregnant women, and the effects of IGF-I (from 3.12 to 100 ng/mL) was assessed in 3 primary cultures on their passage zero obtained from the same number of umbilical cords for each group. In addition, the effect of IGF-I was studied in the NT group in the presence of picropodophyllin (PPP), a specific inhibitor of IGF-I signaling. Circulating and secreted VEGF and sFlt-1 levels were determined by ELISA. Regarding the effect of IGF-I upon VEGF secretion, it was not possible to detect it by the kit used. **Results:** Serum umbilical cord VEGF levels were not significantly different between the PE and the NT groups (392 ± 87 vs 430 ± 153 pg/mL, $P=0.505$). However, sFlt-1 levels were significantly higher in the PE group as compared with the NT group (1049 ± 545 pg/mL vs 565 ± 125 pg/mL, $P<0.001$). In addition, VEGF/sFlt-1 ratio was significantly lower in the PE group ($P<0.001$). IGF-I stimulates significantly sFlt-1 secretion by HUVEC at 12.50 and 25.00 ng/mL in the NT group ($P<0.005$). However, this stimulatory effect was not observed in the PE group. As compared with the NT group, sFlt-1 secretion was significantly higher ($P<0.05$) in the PE group in cultures treated with vehicle or 3.12 ng/mL IGF-I, without significant changes between groups when cultures were treated with the other doses tested. The presence of PPP significantly abolished the stimulatory effect of IGF-I upon sFlt-1 secretion ($P<0.001$). **Conclusions:** Serum umbilical cord sFlt-1 levels and VEGF/sFlt-1 are higher and lower, respectively, in preeclampsia. On the other hand, IGF-I stimulates significantly the secretion of sFlt-1 by HUVEC in the NT group. The absence of a stimulatory effect of IGF-I upon sFlt-1 secretion in the PE group may be due to the higher basal levels of this soluble receptor in preeclampsia.

Sources of Research Support: CONACYT (Mexico) grant CB-2008-01 Ref. 105151.

Nothing to Disclose: AO, LD, DB, EA, FL, AH

Pub # P3-166

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Uterus, Placenta & Pregnancy, Including Endometriosis (1:30 PM-3:30 PM)

Title Raf Kinase Inhibitor Protein (RKIP) Expression in Human Placenta and Evidence of a Role in Regulating Cytotrophoblast Migration through MAPK, NF[kappa]B and GRK2 Signaling

Author String P Ciarmela, D Marzioni, MS Islam, T Lorenzi, SR Giannubilo, P Stortoni, G Grechi, AL Tranquilli, T Todros M Castellucci
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Body Raf kinase inhibitor protein (RKIP) regulates growth and differentiation signaling pathways and suppresses cancer metastasis. It mediates mitogen-activated protein kinases (MAPK) pathway and accounts for its current name for the ability to bind activated Raf. Its binding to activated Raf results in its inactivation and in ERK phosphorylation. RKIP is also involved in other signaling pathways, as GRK2 and NF-kappaB. Cytotrophoblast differentiation is mediated by MAPK pathway, which is essential for normal placental development. GRK2 is also considered to play a role in cell migration, while impaired trophoblast migration is partly due to elevated PAI expression consequently to NF-kappaB pathway activation. After having investigated RKIP localization in placentas from normal pregnancies and from pregnancies complicated by preeclampsia, we tested if it regulates cytotrophoblast migration. RKIP was identified in all placentas considered in this study and was predominantly expressed in the cytotrophoblast. It was also expressed in HTR-8/SVneo cells, a first trimester cytotrophoblast cell line. Locostatin, a chemical compound able to block RKIP, impaired HTR-8/SV neo cells motility in wound closure experiments; in other experiments locostatin induced ERK phosphorylation, demonstrating the disruption of MAPK signalling. We also documented the presence of GRK2 mRNA and the reduction of phosphorylated RKIP expression by locostatin and the induction of PAI mRNA expression in HTR-8/SV neo cells, suggesting the involvement of GRK2 and NF-kappaB pathways. In conclusion, RKIP is a novel factor expressed in cytotrophoblast cells where it likely regulates cell migration.

Nothing to Disclose: PC, DM, MSI, TL, SRG, PS, GG, ALT, TT, MC

Pub # P3-167

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Uterus, Placenta & Pregnancy, Including Endometriosis (1:30 PM-3:30 PM)

Title *Cyp11a1* Overexpression in Transgenic Mice Leads to Mis-Regulated Progesterone Production and Pregnancy Failure

Author String Y Chien, W-C Cheng, B-c Chung
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Body The cytochrome P450 side-chain cleavage enzyme, P450_{scc} (*CYP11A1*) catalyzes the first and rate-limiting step in steroid biosynthesis. *CYP11A1* overexpression is associated with predisposition to breast cancer, endometrial cancer and polycystic ovary syndrome. We generated *Cyp11a1* transgenic mice to characterize the pathophysiological roles of over-expressed *Cyp11a1*. The transgene contained complete mouse *Cyp11a1* in a bacterial artificial chromosome to recapitulate the endogenous expression of *Cyp11a1* but at the two to three-fold level. *Cyp11a1* transgenic females displayed reduced pregnancy rate, decreased litter size *in utero*, delayed parturition and no live birth. The transgenic females produced less serum progesterone (P4) during early pregnancy; the progesterone level returned to normal afterwards, but did not decrease near term as the wildtype did. Pregnant *Cyp11a1* transgenic females could not support normal implantation and placentation at the correct time, and more embryos were absorbed. The ectoplacental cone (EPC) in the decidua of pregnant transgenic females was also abnormal. The luteal cells in transgenic ovary had foaming appearance during early pregnancy and gene expression patterns examined by quantitative-RT-PCR were perturbed. Taken these observations together, we conclude that the loss of successful pregnancy in *Cyp11a1* transgenic mice is probably due to aberrant steroid production, abnormal ovarian and uterine functions, anomalous placenta formation and parturition problem.

Nothing to Disclose: YC, W-CC, B-CC

Pub # P3-168

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Uterus, Placenta & Pregnancy, Including Endometriosis (1:30 PM-3:30 PM)

Title Cell Membrane and Cytosolic Calcium Transporters Are Expressed and Induced in Human Preeclamptic Placental Tissue

Author String H Yang, E-B Jeung
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Body The ionic form of Ca^{2+} serves as a universal intracellular messenger to modulate many processes such as neurotransmission, enzyme and hormone secretion as well as many biological processes, i.e. cell cycle regulation and programmed cell death. The transepithelial Ca^{2+} active transport proceeds through a well-controlled sequence of events consisting of members of the transient receptor potential (TRPV5 and 6) gene family, and then cytosolic diffusion of Ca^{2+} bound to calcium binding proteins (CaBP-9k/-28k) and basolateral extrusion of Ca^{2+} through plasma membrane Ca^{2+} -ATPase 1 (PMCA1) and finally to a lesser extent by $\text{Na}^+/\text{K}^+/\text{Ca}^{2+}$ exchanger (NCKX3). Similar mechanisms of transcellular Ca^{2+} transport exist in renal distal tubules and syncytiotrophoblasts of the placenta. In this study, the expression of cell membrane and cytosolic calcium transporters was shown in dissected three sections (fetal-, central-, maternal-) of preeclamptic placenta of human. During preterm labor of human, placental expressions of calcium transporters, i.e., TRPV6, PMCA1, NCKX3, were increased at transcriptional and translational levels in preeclamptic placenta (PEP) at fetal and maternal sections. However, the expression levels of CaBP-28k mRNA and protein were decreased in PEP at fetal and maternal sections compared to those of normal placenta (NP). Taken together, these results indicate that placental calcium transporters play potential roles in differential sections of placenta between NP and PEP, suggesting that induced calcium transporters of PEP may be involved in preeclamptic stress in human placenta, which is a determinant factor affecting calcium transfer in preeclamptic placental tissues.

Nothing to Disclose: HY, E-BJ

Pub # P3-169

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Uterus, Placenta & Pregnancy, Including Endometriosis (1:30 PM-3:30 PM)

Title Expression of Calcium Transport Genes in Maternal-Fetal Placenta of Mice

Author String T-H Koo, E-B Jeung
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Body During pregnancy, the placenta represents the establishment of an intimate connection between mother and fetus that is specific to mammals. Many types of calcium channel, intracellular calcium binding proteins, Na^+/Ca^{2+} exchangers (NCX), transient receptor potential cation channels (TRPV) were found in placenta. In this study, the calcium channel in maternal-fetal Ca^{2+} transport was investigated using the phenotypes of wild-type, calbindin-D_{9k} (CaBP-9k), D-28k (CaBP-28k) single knockout (KO) and CaBP-9k/28k double KO mouse models. Expressions of calcium transport genes in three dissected sections of placenta (MP: maternal-, CP: central-, FP: fetal-) were examined at gestational day 19 (GD 19) in these mice. The expression of TRPV6 mRNA and protein was highest in CaBP-28k KO mice at MP and CP compared with other KO mice, or CaBP-9k KO mice at FP. And the level of CaBP-9k was significantly induced in CaBP-28k KO mice at MP and CP, but not altered at FP. In addition, the expression of CaBP-28k mRNA and protein were reduced in CaBP-9k KO mice than WT in all sections of placenta. These results indicate that TRPV6 participates in transferring calcium ions between maternal and fetal compartments and alteration of CaBP-9k/28k is involved in intracellular Ca^{2+} buffering system among single and double KO mice. Taken together, TRPV6 and CaBP-9k gene may play a role as a key element in controlling calcium transport in the placenta during pregnancy.

Nothing to Disclose: T-HK, E-BJ

Pub # P3-170

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Uterus, Placenta & Pregnancy, Including Endometriosis (1:30 PM-3:30 PM)

Title Expressions of Apoptosis-Related Genes and Endoplasmic Reticulum Stress in the Uterus of *Calbindin-D9k* and *-D28k* Knockout Mice

Author String E-M Jung, E-B Jeung
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Body Calcium (Ca^{2+}) is known to be an important regulator in apoptotic signaling. Calbindin-D_{9k} (CaBP-9k) and -D_{28k} (CaBP-28k) have a high affinity to Ca^{2+} ions. Uterine calbindins appear to be involved in the regulation of myometrial activities by intracellular Ca^{2+} . In addition, uterine calbindins are expressed in the mouse endometrium and strictly regulated by steroid hormones during implantation and development. The aim of the present study was to evaluate the regulation of apoptosis in the uterus of immature CaBP-9k, CaBP-28k, and CaBP-9k/28k knockout (KO) mouse models. Our finding indicated that Bax protein levels were enhanced in the uterus of CaBP-28k and CaBP-9k/28k KO mice compared to wild-type (WT) mice, but no difference was observed in Bcl-2 protein. Also, the levels of caspase 3, 6 and 7 proteins were induced in both CaBP-28k and CaBP-9k/28k KO mice compared to WT mice. These results suggest that absence of CaBP-28k may induce an increase in the apoptotic signaling. We further investigated the expressions of the endoplasmic reticulum stress genes by Western blot analysis in calbindin KO mice. CHOP and Bip proteins were induced in CaBP-28k KO mice compared to WT mice. When immature mice were treated with 17beta-estradiol (E2) and progesterone (P4) for three days, the expression levels of Bax and caspase 3 proteins increased following treatment with E2 in WT and CaBP-9k KO mice, and P4 in CaBP-28k KO mice. Based on these results, the expression of CaBP-28k blocked up-regulation of apoptosis-related and ER stress genes, implying that CaBP-28k may decrease the apoptosis-related and ER stress genes in uterine tissues of mice.

Nothing to Disclose: E-MJ, E-BJ

Pub # P3-171

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Uterus, Placenta & Pregnancy, Including Endometriosis (1:30 PM-3:30 PM)

Title Expression of *Calbindin-D_{28k}* Is Inversely Correlated with Proapoptotic Gene Expression in Hydrogen Peroxide-Induced Cell Death in Endometrial Cells

Author String E-M Jung, E-B Jeung
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Body Calbindin-D_{28k} (CaBP-28k) is a calcium binding protein important for intracellular Ca²⁺ buffering and known to have anti-apoptotic properties in neurons, osteoblasts and male germ cells. Although endometrial cancer is a common invasive gynecologic malignancy, the involvement of uterine CaBP-28k in apoptotic signaling of endometrial cancer is poorly understood. The present study investigated the role of CaBP-28k in hydrogen peroxide (H₂O₂)-induced apoptotic signaling in human endometrial Ishikawa cells. The dose-dependent and time-dependent effect of H₂O₂ on Bax, p53 and Bcl-2 expression was assessed by Western blot analysis. Treatment of the cells with 1 mM H₂O₂ for 1 h induced an increase in Bax and p53 expression, but the expression of Bcl-2 was not affected by H₂O₂ treatment. Interestingly, over-expression of CaBP-28k inhibited cell death and caused a decrease in Bax, p53 and caspase 3 expression during H₂O₂-induced apoptosis, suggesting that CaBP-28k may block the up-regulation of apoptosis-related gene expression. SiRNA knockdown of CaBP-28k resulted in an elevation of H₂O₂-induced cell death and an increase in Bax, p53 and caspase 3, providing additional evidence that induction of CaBP-28k gene might be associated with survival signaling during H₂O₂-mediated cell death. Overall, these results suggest that CaBP-28k expression may be inversely correlated with pro-apoptotic gene expression in human endometrial Ishikawa cells.

Nothing to Disclose: E-MJ, E-BJ

Pub # P3-172

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Uterus, Placenta & Pregnancy, Including Endometriosis (1:30 PM-3:30 PM)

Title Aberrant Expression of IAP Family in Endometriotic Cells May Cause Resistance to Apoptosis

Author String F Taniguchi, M Izawa, T Uegaki, T Iwabe, N Terakawa, T Harada
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Body

Aim:Decreased susceptibility of endometrial tissue to apoptosis may contribute to the pathogenesis of endometriosis. Inhibitor of apoptosis protein (IAP) family may be closely linked to the development of endometriosis. In our previous study, we revealed that TNF α is elevated in peritoneal fluid of women with endometriosis, and promotes mitogenic activity in endometriotic cells. The aim of present study was to determine the difference of IAP family expression between ovarian endometriomas and eutopic endometrial tissues. Subsequently, we evaluate the effect of TNF α on IAP family expression in cultured stromal cells derived from these tissues.

Materials and Methods:Ectopic endometrial tissue from ovarian endometriomas and matched eutopic endometrial tissue samples were collected from premenopausal women. Eutopic endometrial tissues with benign gynecologic diseases and no evidence of endometriosis were used as control. Ectopic or eutopic endometrial stromal cells (ESCs) were isolated from these tissues. Staurosporine (SS:0.5[μ]M) and TNF α (0.1ng/ml) were used as an apoptosis inducer and an inflammatory mediator, respectively. Gene and protein expression patterns for IAP family, cIAP-1(*birc2*), cIAP-2(*birc3*), XIAP(*birc4*), and survivin(*birc5*) were evaluated by real-time RT-PCR and immunohistochemical staining. Number of surviving cells and activation of caspases were assessed by WST-8 assay, Annexin-V staining and immunoblotting.

Results:All four IAP family mRNAs were more intensively expressed in endometriotic tissues derived from ovarian endometrioma compared with those of eutopic endometrial tissues. Interestingly, IAP family mRNA expression in eutopic endometrial tissues of women with ovarian endometriomas was higher than control. Both epithelial and stromal cells of ovarian endometriotic tissues exhibited higher protein expression of these four IAPs than eutopic endometrial tissues. After induction of apoptosis, 55% of eutopic ESCs survived versus 70% of ectopic ESCs. Procaspace-3 or -7 was more activated by SS in eutopic than in ectopic ESCs. Survivin silencing in SS-treated ectopic ESCs led to activation of caspases and increase of apoptotic cells. Following TNF α addition, cIAP-2 mRNA expression in ectopic ESCs was remarkably enhanced compared with eutopic ESCs.

Conclusion:Aberrant expression of IAP family in ovarian endometriomas may sustain their abnormal survival in ectopic sites. IAP family would be critically involved in pathogenesis of endometriosis.

Sources of Research Support: Ministry of Education, Science, Culture, Sports and technology of Japan.

Nothing to Disclose: FT, MI, TU, TI, NT, TH

Pub # P3-173

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Uterus, Placenta & Pregnancy, Including Endometriosis (1:30 PM-3:30 PM)

Title Progesterone-Dependent Endometrial Stromal Cell Decidualization Requires Steroid Receptor Coregulator-2

Author String R Kommagani, MM Szwarc, J Jeong, HL Franco, CJ Creighton, FJ DeMayo, JP Lydon, BW O'Malley
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Body Ovarian estrogen (E2) and progesterone (P4) are indispensable for embryo-implantation and endometrial stromal decidualization; however, the molecular mechanisms that underpin these reproductive processes are unclear. Steroid receptor coregulator-2 (SRC-2) belongs to the multifunctional SRC/p160 family which also includes SRC-1 and SRC-3. Sharing strong sequence homology, all three SRCs exert diverse regulatory effects by modulating the transcriptional potency of nuclear receptor family members, including the estrogen and progesterone receptor (ER and PR respectively). Importantly, absence of SRC-2 in PR positive cells in the epithelial, stromal, and myometrial compartments of the murine uterus results in a striking infertility defect. This reproductive phenotype highlights a key role for SRC-2 in uterine function which is not shared with other coregulators. Intriguingly, abrogation of uterine SRC-2 does not block embryo apposition or attachment to the apical surface of luminal epithelial cells of the endometrium but rather prevents P4-dependent local decidualization of the sub-epithelial stroma. Remarkably, epithelial-specific ablation of SRC-2 in the murine uterus does not compromise endometrial functionality, again underscoring the unique importance of stromal derived SRC-2 in uterine function. The stromal decidualization defect resulting from SRC-2 ablation is reflected at the molecular level by a marked attenuation in P4 responsive target genes known to be critical for P4 dependent decidualization. Conversely, the induction of E2 or P4 target genes involved in embryo implantation (i.e. leukemia inhibitory factor (LIF) and Indian hedgehog (Ihh) respectively) is not affected by SRC-2's absence. As with mouse studies, decidualization of primary human stromal cells (HESCs) in culture is blocked by SRC-2 knockdown; however, HESC decidualization is unaffected by knockdown of SRC-1 or SRC-3. As a consequence of SRC-2 knockdown, molecular studies disclose a striking decrease in the induction of a subset of P4 target genes (i.e. WNT4 and FKBP5) which are essential for the stromal-epithelioid transformation step, the cellular hallmark of endometrial decidualization. Collectively, these studies not only showcase the evolutionary importance of SRC-2 in endometrial biology but also suggest that deregulation of this coregulator may underpin a spectrum of hormone-dependent uterine pathologies such as endometriosis and endometrial cancer.

Sources of Research Support: NIH grant U54 HD-07495.

Nothing to Disclose: RK, MMS, JJ, HLF, CJC, FJD, JPL, BWO

Pub # P3-174

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Uterus, Placenta & Pregnancy, Including Endometriosis (1:30 PM-3:30 PM)

Title Analysis of *FOXP3* Polymorphisms in Infertile Women with and without Endometriosis

Author String B Bianco, JS Teles, GM Andre, DM Christofolini, CP Barbosa
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Body
Background: Immunological theories suggest that changes in the immune system could prevent the ability to eliminate the endometrium of the pelvic cavity. In women with endometriosis is possible that changes in immunity mediated by T cells facilitate the implantation of endometrial fragments or cells in ectopic locations. Recent studies have associated the *FOXP3* (Xp11.23) gene with homeostasis of the immune system and the development of autoimmune diseases. It encodes FOXP3 protein which regulates the activation of T cell and functions as a transcriptional repressor and down-regulates cytokine production in T cells. We hypothesized that the *FOXP3* polymorphisms might be involved in the pathogenesis of endometriosis and/or infertility. In this study, we examined five single-nucleotide polymorphisms (SNPs) in the *FOXP3* gene in a group of infertile women with and without endometriosis and controls.
Methods of Study: Case-control study that included 177 infertile women with endometriosis, 71 women with idiopathic infertility and 171 fertile women as controls. *FOXP3* polymorphisms (rs3761549, rs3761548, rs2232368, rs2232366 and rs2280883) were identified by TaqMan PCR. The chi-squared test was used to detect differences in allele and genotype frequencies between patients and controls. Combined genotype of *FOXP3* was assessed by haplotype analysis. A p-value < 0.05 was considered statistically significant.
Results: Single-marker analysis revealed that *FOXP3* rs3761549 was significantly associated with endometriosis (p=0.003). Considering the infertile group without endometriosis, single-marker analysis revealed statistical difference for rs2280883 (p=0.024) and rs2232368 (p=0.034) *FOXP3* polymorphisms. No associations were found considering rs3761548 and rs2232366 either for endometriosis-related infertility group or idiopathic infertility group. Haplotype analysis of five *FOXP3* polymorphisms identified a haplotype "CTTGA" associated with endometriosis (p=0.011) and [ldquo]ACTAG[rdquo] associated with idiopathic infertility (p=0.014).
Conclusion: This is the first study to report an association between *FOXP3* polymorphisms and endometriosis and/or infertility. These findings require replication in other populations but suggest the *FOXP3* polymorphisms can be associated with risk of idiopathic infertility (rs2280883 and rs2232368) and endometriosis (rs3761549) in Brazilian women.

Sources of Research Support: FAPESP granting student Juliana Souto Teles a Scientific Initiation scholarship (FAPESP No.2009/01960-0). Grants No.2009/09980-2 from FAPESP.

Nothing to Disclose: BB, JST, GMA, DMC, CPB

Pub # P3-175

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Uterus, Placenta & Pregnancy, Including Endometriosis (1:30 PM-3:30 PM)

Title Association of *FCRL3* C-169T Promoter Single-Nucleotide Polymorphism in Idiopathic Infertility and Infertility-Related Endometriosis

Author String JS Teles, B Bianco, AM Bentes de Souza, DM Christofolini, CP Barbosa
Faculdade de Medicina do ABC, Santo André, Brazil

Body
Background: Numerous data suggest that aberrant immunologic mechanisms are involved in the pathophysiology of endometriosis. FCRL3 protein is an orphan cell surface receptor with homology to the Fc immunoreceptors and is expressed predominantly in B lymphocytes in lymph nodes and germinal centers. The *FCRL3* C-169T polymorphism alters the expression of *FCRL3* by affecting the binding affinity of nuclear factor-kappa-B (NF-[kappa]B) to the *FCRL3* promoter. Therefore, an increased level of FCRL3 protein, which is correlated with the -169C susceptibility allele, may result in B cell abnormalities and higher levels of autoantibodies. Moreover, considering the endometriosis, there is no doubt that B lymphocyte abnormalities are responsible for producing both specific and non-specific autoantibody to the endometrium, and thus in turn contributes to the pathogenesis of the disease. The present aimed to evaluate a possible relationship between endometriosis and/or infertility and *FCRL3* C-169T polymorphism.
Methods of Study: Case-control studied included 167 infertile women with endometriosis, 60 idiopathic infertile women and 167 fertile women. Detection of *FCRL3* C-169T polymorphism was performed using TaqMan PCR. The chi-squared test was used to detect differences in allele and genotype frequencies between patients and controls, to estimate the Hardy-Weinberg equilibrium and to calculate the power of the test. A p-value < 0.05 was considered statistically significant.
Results: We were able to demonstrate a statistically significant difference in the genotype and allele frequencies of the *FCRL3* C-169T polymorphism between endometriosis-related infertility (p=0.003 and p=0.001) and idiopathic infertility (p=0.027 and p=0.0185) versus controls. When we compared infertile groups with and without endometriosis there was no statistically significant difference considering genotype (p=0.786) and allele (p=1.0) distribution of the studied polymorphism. The power of the test calculated was 0.99 ($\alpha = 0.01$) to endometriosis-related infertility group and >0.90 ($\alpha=0.05$) for idiopathic infertile group. The genotype frequencies were in Hardy-Weinberg equilibrium in all studied groups.
Conclusion: To our knowledge, this is the first study in the literature to investigate the association between *FCRL3* polymorphism and endometriosis and/or infertility. The results suggest that *FCRL3* C-169T polymorphism may play an important role in the pathogenesis of endometriosis and/or infertility.

Sources of Research Support: FAPESP granting student Juliana Souto Teles a Scientific Initiation scholarship (No.2009/01960-0). Grants No.2009/09980-2 from FAPESP.

Nothing to Disclose: JST, BB, AMBdS, DMC, CPB

Pub # P3-176

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Uterus, Placenta & Pregnancy, Including Endometriosis (1:30 PM-3:30 PM)

Title *COMT* Polymorphism and the Risk of Endometriosis-Related Infertility

Author String DM Christofolini, JS Teles, AM Bentes de Souza, B Bianco, CP Barbosa
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Body **Background:** Estrogens are important factors in the development of endometriosis, and can induce cell proliferation and stimulate cell division. *COMT* constitutes a crucial element in estrogen metabolism and has been suggested to be involved in the development of endometriosis. Thus, polymorphisms in gene coding for estrogen metabolic pathways could increase individual estrogen-dependent diseases susceptibility and lead to the indication of individuals at higher risk. The aim of the study was to analyze whether a genetic variation in steroid hormone metabolism gene *COMT* (val/met, rs4680) is associated with endometriosis in infertile women.

Methods of Study: A case-control study that included 198 infertile women with endometriosis, 71 infertile women without endometriosis and 168 fertile women as control group. *COMT* (val/met) genotypes were identified by real time PCR (genotyping TaqMan assay). The chi-squared test was used to detect differences in allele and genotype frequencies between patients and controls, and to estimate the Hardy-Weinberg equilibrium. A *p*-value < 0.05 was considered statistically significant.

Results: The data showed no statistical difference in the distribution of *COMT* genotypes and alleles neither between infertile patients with endometriosis and control group (*p*=0.567 and *p*=0.920), regardless disease degree, nor between infertile patients without endometriosis and control group (*p*=0.460 and *p*=0.522). The endometriosis-related infertility, idiopathic infertility and control groups were in Hardy-Weinberg equilibrium.

Conclusion: In conclusion, the *COMT* val/met polymorphism is not associated to endometriosis-related infertility in the Brazilian population evaluated. However, more studies in larger populations are necessary to confirm these results.

Keywords: endometriosis, infertility, polymorphism, estrogen hormone, *COMT*.

Nothing to Disclose: DMC, JST, AMBdS, BB, CPB

Pub # P3-177

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Uterus, Placenta & Pregnancy, Including Endometriosis (1:30 PM-3:30 PM)

Title Analysis of Vitamin D Receptor Gene (*VDR*) Polymorphisms in Women with and without Endometriosis

Author String FL Vilarino, B Bianco, TG Lerner, JS Teles, FA Mafra, DM Christofolini, CP Barbosa
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Body

Introduction: An aberrant immunologic mechanism has been suggested to be involved in the pathogenesis of endometriosis. Genetic alterations in vitamin D receptor gene (*VDR*) may lead to important defects in gene activation affecting principally immune function. The function of the *VDR* gene is influenced by several genetic polymorphisms which are associated with a susceptibility to a range of autoimmune diseases. We have hypothesized a possible relationship between endometriosis and/or infertility and the *VDR* polymorphisms (*ApaI*, *TaqI*, *FokI* and *BmsI*).

Methods: Case-control study that included 132 women with endometriosis-related infertility, 62 women with idiopathic infertility and 133 controls. *VDR* polymorphisms were studied by restriction fragment length polymorphism (RFLP-PCR). The chi-square was used to compare allele and genotype frequencies between groups and to estimate the Hardy-Weinberg equilibrium. Combined genotype of *VDR* was assessed by haplotype analysis. A *p*-value < 0.05 was considered statistically significant.

Results: We found relatively similar *VDR* polymorphisms genotype frequencies in cases and controls and we did not observe any associations between polymorphisms in *ApaI*, *TaqI*, *FokI* and *BmsI* and endometriosis risk in endometriosis-related infertility or idiopathic infertile groups. When the patients with minimal/mild endometriosis and moderate/severe endometriosis were studied separately, no difference was also found for any *VDR* polymorphism. When we compared infertile groups with and without endometriosis there was no statistically significant differences related to the studied polymorphism frequency. Haplotype analysis showed that none of the *VDR* haplotypes were associated to endometriosis-related infertility sample. Statistical analyses showed that the genotype distribution in endometriosis-related infertility, idiopathic infertile and control groups for all polymorphisms studied were in Hardy-Weinberg equilibrium.

Conclusion: The results suggest that *VDR* (*ApaI*, *TaqI*, *FokI* and *BmsI*) polymorphisms play not a role in the pathogenesis of idiopathic infertility and endometriosis-related infertility in Brazilian women. However, it does not exclude the role of vitamin D in the endometriosis. Maybe other genetic alteration can act in the vitamin D influences the disease.

Sources of Research Support: FAPESP granting student Tatiana Goberstein Lerner (2010/01104-6) and Juliana Souto Teles (2009/01960-2) a Scientific Initiation scholarship. Grants No. 2010/00459-5 from FAPESP.

Nothing to Disclose: FLV, BB, TGL, JST, FAM, DMC, CPB

Pub # P3-178

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Uterus, Placenta & Pregnancy, Including Endometriosis (1:30 PM-3:30 PM)

Title Up-Regulation of Hydroxysteroid 17 β -Dehydrogenase 6 (HSD17B6) and Down-Regulation of HSD17B2 in Peritoneal, Deep and Ovarian Endometriosis

Author String K Huhtinen, A Salminen, M Yli-Heikkilä, H Kujari, P Suvitie, M Setälä, P Harkki, J Jalkanen, J Fraser, A Perheentupa, M Poutanen
Institute of Biomedicine, University of Turku, Turku, Finland; Turku University Hospital, Turku, Finland; Turku University Hospital, Turku, Finland; Päättälä Central Hospital, Lahti, Finland; Helsinki University Hospital, Helsinki, Finland; North Karelia Central Hospital, Joensuu, Finland

Body Endometriosis is a complex disease characterized by the presence of endometrial glands and stroma outside the uterine cavity. Estrogens and progesterone regulate the cellular proliferation and differentiation of the endometrium while in endometriosis the hormone action may be dysregulated, leading to the increased proliferation of endometriosis cells. However, the data on the expression of estrogen metabolizing enzymes e.g. hydroxysteroid (17 β) dehydrogenases (HSD17Bs) in different types of endometriosis lesions is inconclusive. In this study, we have evaluated the expression of all presently described HSD17Bs (types 1-14) in peritoneal, deep and ovarian endometriosis as well as in eutopic endometrium of endometriosis patients and healthy controls. The data shows that the expression of HSD17B2 and HSD17B6 present the most marked differences between the healthy endometrium and endometriosis tissue. The level of HSD17B2 mRNA in the secretory phase peritoneal, ovarian and deep endometriosis was 0.13-0.15 fold ($p < 0.05$) as compared to the healthy endometrium. No differences were detected in the proliferative phase. Interestingly, the expression of HSD17B6 was highly increased in endometriosis, particularly in deep lesions (6.7 and 8.2 -fold in proliferative and secretory phase, respectively). In the endometrium of healthy controls, the mRNA expression of HSD17B2 and HSD17B6 correlated with serum progesterone concentration, while in the patient endometrium the correlations were diminished and totally lost in endometriosis lesions. As HSD17B2 and HSD17B6 metabolize a variety of steroidal substrates, the aberrant expression of these enzymes in the different types of endometriosis may be involved in several pathophysiological mechanisms of the disease.

Sources of Research Support: Academy of Finland, TEKES-the Finnish Funding Agency for Technology and Innovation, Sigrid Juselius Foundation.

Nothing to Disclose: KH, AS, MY-H, HK, PS, MS, PH, JJ, JF, AP, MP

Pub # P3-179

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Uterus, Placenta & Pregnancy, Including Endometriosis (1:30 PM-3:30 PM)

Title Metformin Prevents Insulin-Induced Cellular Proliferation and Activation of Signaling Pathways in Endometrial Epithelial Cells

Author String CA Flannery, DJ Selen, HS Taylor
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Body Obesity and Type 2 Diabetes are independent risk factors for the development of endometrial hyperplasia and carcinoma. Insulin resistance and hyperinsulinemia may play a role in endometrial cell proliferation. In several case reports, metformin has reversed progestin-resistant endometrial hyperplasia. We sought to determine the effect of insulin on signaling pathways associated with hyperplasia in primary endometrial epithelial cells. Epithelial cells were separated from the endometrial tissues of seven women, and cultured in low glucose DMEM. Western blot analysis of phosphorylated and total AKT, 4E-BP, and p70S6K was performed after acute insulin stimulation for each group of epithelial cells. We then examined the relative concentrations of the same signaling proteins in the presence of metformin, with and without insulin, in Ishikawa cells. Ishikawa cells are a well-characterized endometrial adenocarcinoma cell line with a PTEN deficiency resulting in unregulated AKT and mTOR activation. Primary epithelial cells demonstrated over a four fold increase ($p=0.02$) in cell proliferation in 24 hours in the presence of insulin relative to vehicle. Insulin induced a robust dose-dependent phosphorylation of AKT in all seven primary epithelial cell cultures, and activation of mTOR signaling in half of them by thirty minutes. This activation was inhibited by wortmannin, a PI3 kinase inhibitor. Metformin treatment of Ishikawa cells reduced insulin-induced cell proliferation by over 40%. Baseline and insulin-induced activation of 4E-BP and p70S6K in Ishikawa cells was also inhibited by metformin. We have demonstrated that human endometrial epithelial cells are sensitive to insulin, which likely promotes cell proliferation through the AKT and mTOR pathways. Our data also provides preliminary evidence that metformin may have a direct effect on the endometrium, resulting in a reduced effect of insulin on cell proliferation and activation of signaling pathways. More than 60% of endometrial cancers have constitutive AKT and mTOR activation from a PTEN deficiency, similar to the Ishikawa cells. Metformin may be beneficial in the treatment of endometrial hyperplasia for women with insulin resistance and hyperinsulinemia.

Nothing to Disclose: CAF, DJS, HST

Pub # P3-180

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Uterus, Placenta & Pregnancy, Including Endometriosis (1:30 PM-3:30 PM)

Title Prostaglandin Pathway and Cortisol Metabolism Alterations in Endometriosis

Author String D Monsivais, J Bray, S Bulun
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Body Endometriosis is an estrogen-dependent disease affecting 5-10% of women of reproductive age; it is characterized by the presence of endometrial tissue outside of the uterine cavity and is associated with chronic pelvic pain, dysmenorrhea and infertility. To identify altered gene expression patterns in endometriosis, we performed gene expression profiling in 8 matched eutopic and ectopic endometrium samples from women with endometriosis. We show that the prostaglandin and cortisol synthesis pathways are altered in endometriosis relative to the normal eutopic endometrium. After microarray validation, we show that in the ectopic endometrium, the gene expression levels of the phospholipase-encoding genes, PLA2G2 and PLA2G5, are elevated by 900- and 600-fold, respectively. The gene expression levels of the prostacyclin gene, PTGIS, and of the prostaglandin receptor genes, PTGER1 and PTGER3, are also significantly elevated in the ectopic endometrium. Thus, the pro-inflammatory microenvironment observed in women with endometriosis, characterized by increased PGE2 levels in the peritoneal fluid, likely results from the overexpression of the genes in the prostaglandin pathway. We also report for the first time, that the cortisol metabolism pathway is altered in endometriosis and that this is affected by the pro-inflammatory milieu of the lesion. We found that the gene encoding the cortisol converting enzyme, HSD11B1, is significantly increased in endometriosis relative to the normal endometrium, whereas the cortisol inactivating enzyme, HSD11B2, is significantly decreased. Correspondingly, the gene expression of the glucocorticoid receptor (GR), which binds cortisol, is also significantly upregulated in endometriosis versus the normal endometrium. We found that treating endometriotic stromal cells with TNF α significantly increases the expression of HSD11B1 and GR, but decreases the expression of HSD11B2. This suggests that the pro-inflammatory milieu in endometriosis contributes to the altered expression of HSD11B1 and HSD11B2 that we observed. It is likely that this alteration contributes to a local increase in cortisol availability to regulate the inflammation associated endometriosis.

Nothing to Disclose: DM, JB, SB

Pub # P3-181

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Uterus, Placenta & Pregnancy, Including Endometriosis (1:30 PM-3:30 PM)

Title A Hypomethylated CpG Island in the Aromatase Gene May Cause Up-Regulation in Endometriotic Cells

Author String M Izawa, F Taniguchi, T Iwabe, N Terakawa, T Harada
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Body
Background: We demonstrated a marked up-regulation of aromatase mRNA expression in endometriotic cells(1). In contrast, a marginal level of aromatase transcript was demonstrated in eutopic endometrial cells. Interestingly, treating endometrial cells with the demethylating agent 5-aza-2'-deoxycytidine led to the aromatase mRNA induction. The unmethylated CpG Island may be related to the up-regulation of aromatase gene transcription.
Objective: We predict an unmethylated CpG Island in endometriotic cells and evaluate the activity as *cis*-acting element.
Patients: Endometrial tissues were obtained from premenopausal women who underwent hysterectomy for fibromas. The chocolate cyst lining in ovaries of patients with endometriosis was the source of endometriotic tissue.
Methods: Stromal cells were prepared from endometrial and endometriotic tissues. Interaction of *trans*-acting factors with the CpG sequence was assessed using EMSA and ChIP assay. Activity of CpG sequence as *cis*-acting element was evaluated using a conventional Luciferase assay. Effect of methylation on the *cis*-activity was examined using a CpG-free Luciferase expression construct.
Results: We searched for CpG islands within the aromatase gene. Although no CpG Island was found within the promoter-proximal region, a CpG Island located at 20 kb upstream from Exon II was predicted. Sequence analysis of bisulphite-treated DNA demonstrated a stretch of CpG-demethylation within the upstream half of the CpG Island in endometriotic cells. In endometrial cells, the upstream half was hypermethylated, and was recognized by the methyl-CpG-binding proteins, MBD1 and MeCP2. The downstream half was hypomethylated in both endometrial and endometriotic cells. Nuclear factor(s) from these cells specifically associated with the CpG island sequence *in vitro*. The upstream half of CpG Island showed an enhancer-like activity, while the downstream half behaved as a silencer-like element. Methylation of the upstream half decreased the enhancer-like activity.
Conclusion: We demonstrated an enhancer-like and a silencer-like element within a predicted CpG Island located at a promoter-distal region in aromatase gene. The finding that methylation suppressed the enhancer-like activity of the CpG Island suggests its role as *cis*-acting element in endometriotic cells. Up-regulation of aromatase gene in endometriosis may be ascribed to the epigenetic disorder associated with the aberrant DNA demethylation.

(1) Izawa M et al., Fertil Steril 2008; 89: 1390

Nothing to Disclose: MI, FT, TI, NT, TH

Pub # P3-182

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Uterus, Placenta & Pregnancy, Including Endometriosis (1:30 PM-3:30 PM)

Title Mitogen-Activated Protein Kinase Mediates Morphological Differentiation and Expression of Vascular Endothelial Growth Factor and Interleukin-11 in Decidualized Human Endometrial Cells

Author String D Zilberman, D Cole, A Wojtczuk, G Weiss, L Goldsmith
UMDNJ-New Jersey Medical School, Newark, NJ

Body Uterine decidualization is required for implantation. Understanding of the mechanisms regulating this differentiation process is limited. Recent data demonstrate PKA-independent aspects involving MAPK are involved. To improve understanding the biochemistry of decidualization, we tested the hypothesis that both morphological differentiation and expression of VEGF and IL-11 are mediated by MAPK. A well characterized line of telomerase-immortalized human endometrial stromal cells which undergo the characteristic decidualization response to increased intracellular cAMP was used an in-vitro model. Replicate wells of cells were incubated in phenol red-free media in the absence (control) or presence of 8-br-cAMP (0.5 mM) (to decidualize the cells) for at least 9 days. To determine the role of MAPK in decidualization, cells were also incubated with 8-br-cAMP in the presence of a specific inhibitor of MAPK activity, UO126. Media were replenished every 48 hours. At the end of the experiments, media levels of IL-11 and VEGF from each replicate well were measured using specific ELISAs. Data are expressed as mean (pg/ml±SEM), n=2 experiments, each in triplicate. Cell morphology was assessed under phase contrast microscopy by two independent examiners and graded on a 1 to 3 scale where 1=the fibroblast phenotype, 3=the decidualized phenotype, and 2=an intermediate phenotype. 4 to 5 replicate fields from each well were examined. Control cells incubated in the absence of 8-br-cAMP consistently exhibited type 1 fibroblast-like morphology and in distinct contrast, cells incubated with 8-br-cAMP demonstrated the characteristic type 3 decidual morphology. Cells incubated with addition of MAPK inhibitor to 8 br-cAMP consistently revealed the intermediate phenotype with grade 2 morphology. 8-br-cAMP increased media VEGF levels 5 fold above those of controls (7323±530 and 1420±300 respectively). Addition of the MAPK inhibitor decreased media levels of VEGF 2 fold below levels from cells incubated with 8-br-cAMP alone (3657±264 and 7323±530 respectively). 8-br-cAMP increased media IL-11 levels 80 fold above those of controls (9012±553 and 113±7 respectively). Addition of the MAPK inhibitor decreased media levels of IL-11 3 fold below levels in cells incubated with 8-br-cAMP alone (2823±444 and 9012±553 respectively). These data support a novel role for MAPK in the morphological changes as well as in IL-11 and VEGF expression during decidualization of human endometrial cells.

Nothing to Disclose: DZ, DC, AW, GW, LG

Pub # P3-183

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Female Reproductive Physiology: Uterus, Placenta & Pregnancy, Including Endometriosis (1:30 PM-3:30 PM)

Title Maternal Hepatic Growth Response to Pregnancy in the Mouse

Author String G Dai, JJ Bustamante, A Myronovych, Y Zou, S Kumar, L Bao, MJ Soares
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Body Pregnancy is characterized by physiological adjustments in the maternal compartment. In this investigation, the influence of pregnancy on maternal liver was examined in CD-1 mice. Dramatic changes were observed in the size of the maternal liver during pregnancy. Livers doubled in weight from the nonpregnant state to day 18 of pregnancy. The pregnancy-induced hepatomegaly was a physiological event of liver growth confirmed by DNA content increase and detection of hepatocyte hyperplasia and hypertrophy. Growth of the liver was initiated following implantation and peaked at parturition. The expression and/or activities of key genes known to regulate liver regeneration, a phenomenon of liver growth compensatory to liver mass loss, were investigated. The results showed that pregnancy-dependent liver growth was associated with IL-6, TNF1 α , NFkB, c-Jun, and IL-1 β , but independent of HGF, FGF1, TNFR1, CAR, and PXR. Furthermore, maternal liver growth was associated with the activation of hepatic STAT3 and β -catenin, but pregnancy did not activate hepatic c-Met and EGFR. The findings suggest that the molecular mechanisms of pregnancy-induced liver growth differ from, but partially share with that of liver injury-induced liver regeneration. In summary, the liver of the mouse accommodates to the demand of pregnancy via a dramatic growth response driven by hepatocyte proliferation and size increase. Remarkably, our findings provide a novel mouse model to investigate the regulation of hepatocyte proliferation, cell size increase, and liver growth.

Nothing to Disclose: GD, JJB, AM, YZ, SK, LB, MJS

Pub # P3-184

Session Information POSTER SESSION: CLINICAL - Female Reproductive Endocrinology (1:30 PM-3:30 PM)

Title Anti Müllerian Hormone Levels in Pre-Pubertal Girls with Duarte Variant Galactosemia Show No Evidence of Premature Ovarian Insufficiency

Author String JR Badik, U Castaneda, TJ Gleason, JB Spencer, C Ficicioglu, K Fitzgerald, JL Fridovich-Keil
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Emory University School of Medicine, Atlanta, GA; Children's Hospital of Philadelphia, University of
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Body Greater than 80 percent of women with classic galactosemia (GG) experience premature ovarian insufficiency (POI). These girls and women with GG also demonstrate unusually low levels of plasma anti-Müllerian hormone (AMH)(1). Duarte galactosemia (DG), a milder variant of galactosemia with an incidence of about 1 in 5000 live births, is approximately 10 times more common than GG. In contrast to infants with GG, newborns with DG demonstrate no potentially lethal acute sequelae and most are lost to follow up in childhood. It is therefore difficult to know if patients with DG are at risk for POI or other long-term complications of galactosemia. To determine if women with DG are at an increased risk for POI, we evaluated plasma AMH levels in girls with classic and Duarte galactosemia in comparison with a control group. Subjects were stratified according to age in the following 3 categories: 0-3 months, 3-18 months, and 18 months-10 years. Plasma samples were collected from consented volunteers and clinical laboratory discards (made available via a HIPAA waiver). Analysis was performed by the Reproductive Endocrine Laboratory at Massachusetts General Hospital (Boston, MA). A total of 159 samples were analyzed (66 control samples, 52 DG patient samples, and 41 GG patient samples). Data from the 3 subject groups were compared by age category, using one-way ANOVA (JMP SAS v. 8.0.1.) with $p \leq 0.05$ considered significant. For the 0-3 month age category, there was no significant difference in AMH levels among the 3 groups (DG vs. GG, $p=0.35$; GG vs. control, $p=0.46$; and DG vs. control, $p=0.66$). In the 3-18 month age category, AMH was significantly lower in the GG group as compared to the DG group ($p=0.0039$) and control group ($p=0.0110$). There was no significant difference in AMH levels between the DG and control groups ($p=0.76$). For the 18 month-10 year-old category, AMH was also significantly lower in the GG group as compared to the DG group ($p=0.0249$) and control group ($p < 0.0001$). There was no significant difference in AMH levels between the DG and control groups ($p=0.2416$). This analysis suggests that unlike girls with GG, girls with DG do not have low pre-pubertal levels of AMH, and therefore are not likely to be at increased risk for POI.

(1)Sanders RD,et al.,Fertil Steril 2009;92:344-51

Sources of Research Support: National Institutes of Health grant RO1 DK059904 awarded to JLFK; National Research Service Award 2 T32 DK007298-31 awarded to JRB.

Nothing to Disclose: JRB, UC, TJG, JBS, CF, KF, JLF-K

Pub # P3-185

Session Information POSTER SESSION: CLINICAL - Female Reproductive Endocrinology (1:30 PM-3:30 PM)

Title Hypoleptinemia in Anorexia Nervosa and Hypothalamic Amenorrhea Is Associated with Symptoms of Depression, Independent of Body Mass Index

Author String EA Lawson, KK Miller, JI Blum, E Meenaghan, M Misra, K Eddy, DB Herzog, A Klibanski
Massachusetts General Hospital, Boston, MA; Massachusetts General Hospital, Boston, MA

Body The fat-derived hormone leptin is anorexigenic, and levels are markedly decreased in subjects with low BMI and high in obesity. Recent data suggest that leptin may also have anxiolytic and anti-depressant properties. In rat models of depression, leptin levels are low and both peripheral and hippocampal administration of leptin improve symptoms. In mice, intraperitoneal administration of leptin ameliorates experimentally induced anxiety-like behaviors. Depression and anxiety are common in women with anorexia nervosa (AN), an illness associated with low weight, body fat and leptin levels. Similarly, there is an increased prevalence of anxiety and depression in normal-weight women with functional hypothalamic amenorrhea (HA), a disorder also characterized by hypoleptinemia. It is unknown whether low leptin levels contribute to the development of mood disorders in these populations. We performed a cross-sectional study to investigate the relationship between leptin levels and symptoms of anxiety and depression in AN and HA. We studied 64 women: 15 AN, 12 normal-weight HA, 20 normal-weight in good health (HC), and 17 overweight or obese (OB) healthy controls. We measured fasting serum leptin levels and administered Hamilton Rating Scales for Anxiety (HAM-A) and Depression (HAM-D). The mean age was 27.7 ± 0.9 yrs and did not differ between groups. Mean body mass index (BMI), %ideal body weight (IBW) and %fat were lowest in AN (BMI 18.3 ± 0.3 , % IBW 79.6 ± 0.8 , %fat 17.4 ± 0.9 , $p < 0.0001$), intermediate in HA and HC, and highest in OB (BMI 31.1 ± 1.1 , % IBW 131.0 ± 4.3 , %fat 36.9 ± 1.2 , $p < 0.0001$). %fat was lower in HA than HC (23.6 ± 0.8 vs. 26.7 ± 1.1 , $p < 0.05$). The months of amenorrhea did not differ between HA and AN. HAM-D scores were higher in AN than other groups, higher in HA than OB, and lowest in HC. HAM-A scores were higher in AN than OB and HC, higher in HA than OB, and lowest in HC. Leptin was lowest in AN (2.8 ± 0.5 , $p < 0.0006$), higher in HC than HA (8.8 ± 0.7 vs. 6.3 ± 0.8 , $p = 0.03$), and highest in OB (23.8 ± 2.8 , $p < 0.0001$). Leptin levels were inversely associated with HAM-D scores ($r = -0.43$, $p = 0.0004$); this relationship remained significant after controlling for BMI. Leptin levels were inversely associated with HAM-A scores ($r = -0.34$, $p = 0.006$); this relationship was not significant after controlling for BMI. We conclude that leptin may mediate symptoms of depression independent of BMI.

Sources of Research Support: Investigator-initiated grant from Bioenvision. NIH grants: M01 RR01066, UL1 RR025758, and R01-DK052625.

Nothing to Disclose: EAL, KKM, JIB, EM, MM, KE, DBH, AK

Pub # P3-186

Session Information POSTER SESSION: CLINICAL - Female Reproductive Endocrinology (1:30 PM-3:30 PM)

Title Effects of Transdermal Testosterone on Natriuretic Peptide Levels in Androgen-Deficient Women with Hypopituitarism

Author String E Lin, E McCabe, TJ Wang, KK Miller
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Body

Context: Natriuretic peptides, which are counterregulatory to the renin-angiotensin-aldosterone system, are 60-80% higher in premenopausal women than in men, and this gender dimorphism may contribute to the lower prevalence of cardiovascular disease and hypertension in women. However, the mechanisms underlying the difference in natriuretic peptide levels are not understood. A large cross-sectional analysis from the Dallas Heart Study demonstrated an inverse association between free testosterone levels and natriuretic peptides in healthy premenopausal women.

Objective: To determine whether physiologic transdermal testosterone replacement alters natriuretic peptide levels in hypoandrogenic women.

Design and Methods: 51 women with acquired hypopituitarism due to hypothalamic-pituitary disorders and hypoandrogenism (mean age 41 ± 7 years, body mass index 28.3 ± 7.3 kg/m²) were randomized to physiologic transdermal testosterone replacement therapy (300 mcg daily) or placebo for three months. N-terminal pro-B-type natriuretic peptide (NT-proBNP) (Elecsys pro BNP Assay, Roche Diagnostics, Indianapolis, IN) and free testosterone levels (equilibrium dialysis; Esoterix Endocrinology, Calabasas Hills, CA) were determined at baseline, one month, and three months. NT-proBNP, the pro form of B-type natriuretic peptide (BNP), is an integrated measure of natriuretic peptide concentrations and is used clinically in heart-failure patients and in hypertension research.

Results: Mean free testosterone increased into the normal female physiologic range in the treatment group and remained low in the placebo group. Over the 3-month study period, NT-proBNP levels significantly decreased in the treatment group compared with the placebo group (repeated measured ANOVA time x treatment interaction $p = 0.009$), with 41% lower levels in the testosterone group compared with placebo at 1 month ($p = 0.005$) and 93% at 3 months ($p = 0.03$). After adjusting for age, body mass index, systolic blood pressure and homeostasis model of assessment-insulin resistance, the decrease in NT-proBNP levels remained significant ($p = 0.008$). The change in NT-proBNP over three months was inversely associated with change in free testosterone (Spearman's $\rho = -0.41$, $p = 0.01$).

Conclusions: Testosterone administration in hypoandrogenic women results in decreased natriuretic peptide levels, suggesting that testosterone may be an inverse regulator of the natriuretic peptide system.

Nothing to Disclose: EL, EM, TJW, KKM

Pub # P3-187

Session Information POSTER SESSION: CLINICAL - Female Reproductive Endocrinology (1:30 PM-3:30 PM)

Title Effects of Oral Contraceptives on Natriuretic Peptide Levels in Women with Hypothalamic Amenorrhea: A Pilot Study

Author String E Lin, SK Grinspoon, TJ Wang, KK Miller
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Body **Context:** Natriuretic peptides, which are important regulators of salt handling and blood pressure and are counterregulatory to the renin-angiotensin-aldosterone system, are 60-80% higher in healthy young women than in men, consistent with a gender dimorphism. However, the effects of gonadal steroids on natriuretic peptides are not understood.

Objective: The objective of our study was to determine whether oral contraceptives (OCPs) alter natriuretic peptide levels in hypoestrogenemic young women.

Design and Methods: 12 otherwise healthy women with functional hypothalamic amenorrhea (mean age 26 ± 6 years, body mass index $19.6 \pm 1.6 \text{ kg/m}^2$, and comparable baseline NT-proBNP levels) were randomized to OCPs or placebo for three months. N-terminal pro-B-type natriuretic peptide (NT-proBNP) (Elecys pro BNP Assay, Roche Diagnostics, Indianapolis, IN) and free testosterone levels (calculated using the laws of mass action; testosterone by AxSYM Testosterone Assay, Abbot Diagnostics, Chicago, IL) were determined at baseline and three months. NT-proBNP, the pro form of B-type natriuretic peptide (BNP), is an integrated measure of natriuretic peptide concentrations used to assess natriuretic peptide status clinically in heart-failure patients and in hypertension research.

Results: Mean NT-proBNP levels increased significantly in the OCP group compared with placebo ($p = 0.0002$). Mean NT-proBNP levels increased from $38.5 \pm 12.0 \text{ pg/mL}$ (50th percentile of the normal range) to $72.1 \pm 23.1 \text{ pg/mL}$ (80th-90th percentile) in the OCP treatment group ($p = 0.05$). There was no change in the placebo group, with mean NT-proBNP levels 30.7 ± 5.6 (40th percentile) at baseline and $27.7 \pm 4.7 \text{ pg/mL}$ (30-40th percentile) at 3 months ($p = 0.19$). There was no change in body mass index ($p = 0.84$), total body water ($p = 0.45$), percent total body water ($p = 0.77$), or blood pressure (systolic blood pressure $p = 0.44$, diastolic blood pressure $p = 0.86$, mean arterial pressure $p = 0.67$) from baseline to three months. At three months, NT-proBNP levels were inversely correlated with free testosterone levels ($R = -0.64$, $p = 0.02$).

Conclusions: Our data demonstrate that NT-proBNP levels increase with OCP administration. These effects may be mediated by estrogens, progestins, or an OCP-related decrease in androgen levels.

Sources of Research Support: In part by an investigator-initiated grant from Ortho-McNeil Pharmaceuticals.

Nothing to Disclose: EL, SKG, TJW, KKM

Pub # P3-188

Session Information POSTER SESSION: CLINICAL - Female Reproductive Endocrinology (1:30 PM-3:30 PM)

Title Compared to Secondary Amenorrhea, Primary Amenorrhea in Patients with Primary Ovarian Insufficiency Is Not Associated with Increased Prevalence of Major Depressive Disorder, yet Is Associated with Lower Estradiol Levels, and Smaller Ovarian and Uterine Size

Author String M Lodish, N Sinaii, T Prodanov, C Ferguson, S Dicker, S Sullivan, V Vanderhoof, L Nelson
National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD; Clinical Center, National Institutes of Health, Bethesda, MD; National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD

Body

Background
We hypothesized that women with 46 XX spontaneous primary ovarian insufficiency (POI) with primary (1 [deg]) amenorrhea would be more likely to have emotional distress later in life related to the diagnosis as compared to those with secondary (2[deg]) amenorrhea.

Objectives
To gain insight into potential approaches to improve care of adolescents and young women diagnosed with POI.

Methods
In 625 women with POI (41 with 1[deg] amenorrhea and 584 with 2[deg] amenorrhea), we administered the PRIME-MD to detect major depressive disorder (MDD), suicidal ideation (SI) and other depressive disorders (ODD). We measured other associated clinical and laboratory parameters related to POI, and estrogen replacement therapies were discontinued for 2 weeks prior to the study visit. Data were analyzed using t-test, Pearson's correlation, and Fisher's exact test.

Results
Women with 1[deg] amenorrhea were younger at the time of their visit to the NIH as compared to women with 2[deg] amenorrhea, 27.3 ± 6.0 yrs vs. 32.4 ± 5.3 yrs ($P < 0.0001$). Women with 1[deg] amenorrhea were equally likely to have MDD (7.7% vs. 8.9%), SI (5.1% vs. 5.3%) and ODD (5.1% vs. 8.7%) when compared to women with 2[deg] amenorrhea. Women with 1[deg] amenorrhea had a lower average estradiol level 30.7 ± 15.4 pg/mL as compared to women with 2[deg] amenorrhea, 51.2 ± 57.0 pg/mL ($P < 0.0001$). Women with 1 [deg] amenorrhea had a smaller total ovarian volume (2.5 ± 4.1 cm³ vs. 5.7 ± 7.9 cm³; $P < 0.0001$). Women with 1[deg] amenorrhea also had a smaller mean uterine craniocaudal dimension (5.9 ± 1.2 vs. 7.0 ± 1.3 cm³, $P = 0.0049$). Women with 1[deg] amenorrhea have had a significantly longer duration of time elapsed between onset of menstrual irregularities and age at study visit (10.2 ± 5.9 yrs vs. 7.3 ± 6.4 yrs ($P < 0.05$).

Conclusions
While it is reassuring that women with 1[deg] amenorrhea do not have an increase in depressive disorders, the fact that women with 1[deg] amenorrhea have significantly lower estradiol levels and a smaller ovarian and uterine craniocaudal diminension has potentially important implications with regard to fertility and raises important questions regarding pathophysiologic mechanism.

Sources of Research Support: NICHD Intramural Research.

Nothing to Disclose: ML, NS, TP, CF, SD, SS, VV, LN

Pub # P3-189

Session Information POSTER SESSION: CLINICAL - Female Reproductive Endocrinology (1:30 PM-3:30 PM)

Title Thyroid and Parietal Cell Autoimmunity Are Associated with a More Severe Reproductive Phenotype in Women with 46,XX Primary Ovarian Insufficiency (POI)

Author String SD Sullivan, D Weinstein, Z Xiongce, V Vanderhoof, T Heller, LM Nelson
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Body Among women with 46,XX POI, reproductive phenotype varies, ranging from overt to occult POI. Women with POI also have increased risk of autoimmunity, suggesting autoimmune factors may affect the degree of ovarian dysfunction. The thymectomized mouse is a model for POI due to autoimmune oophoritis, in which autoantibodies (AB) toward the thyroid (TPO+) and stomach (anti-parietal cell, APA+), as well as to Mater, an oocyte protein, play a role.
HYPOTHESIS: Women with POI who are also TPO+ and/or APA+ have a more severe clinical phenotype.
DESIGN: Retrospective analysis of women with POI evaluated at the NIH from 2000-09 (ages 18-42, N=690). Estradiol (E), FSH, LH, and progesterone (P) were measured by immunoassay; free testosterone (fT) by equilibrium dialysis; AB by ELISA; bone density by DEXA; and ovarian volume by transvaginal ultrasound. Statistics: T-test and Chi-square, P<0.05.
RESULTS: In women with TPO+/APA+ (n=23), E (mean \pm SD) was lower compared to women with only 1 AB+ (TPO+/APA-, n=109; TPO-/APA+, n=41) or neither AB+ (TPO-/APA-, n=513) (31 ± 18 TPO+/APA+ vs. 52 ± 60 TPO+/APA- vs. 50 ± 44 TPO-/APA+ vs. 50 ± 58 pg/mL TPO-/APA-). FSH was higher and fT lower in women with TPO+/APA+ when compared to TPO+/APA- women (FSH 88 ± 32 vs 71 ± 36 IU/L; fT $0.28\pm .14$ vs 0.64 ± 1.5 ng/dL), but neither was different when compared to TPO- women. Compared to TPO- women, TPO+ women also had lower L-spine z-scores (TPO+/APA+ -0.4 ± 1.5 and TPO+/APA- -0.6 ± 1.2 vs TPO-/APA+ 0.1 ± 1.3 and TPO-/APA- -0.15 ± 1.2 , P=.02), an earlier age of diagnosis of POI (30 ± 7 vs 28 ± 7 years, P=0.01), and increased APA autoimmunity (7% vs 17%, P<.001). Compared to APA-, APA+ women had decreased P (0.8 ± 1.5 vs 0.5 ± 0.7 ng/mL, P=.04), decreased fT (0.7 ± 1.4 vs 0.3 ± 0.2 ng/dL, P=.04), and 2x increased incidence of TPO autoimmunity (18% vs 36%, P<.001). Additionally, although not statistically significant, APA+ women had smaller total ovarian volumes compared to APA-, TPO+, and TPO- women (mean 3.7 cc vs 5.5 cc vs 4.8 cc vs 5.5 cc, respectively, P=0.1).
CONCLUSIONS:
Theses data suggest that degree of ovarian dysfunction in POI is related, at least in part, to autoimmunity to the thyroid and to gastric parietal cells. TPO+/APA+ women with POI may represent a distinct etiologic mechanism, possibly related to MATER autoimmunity as reported in the mouse model. Further investigation of this subset of women with POI is warranted.

Nothing to Disclose: SDS, DW, ZX, VV, TH, LMN

Pub # P3-190

Session Information POSTER SESSION: CLINICAL - Female Reproductive Endocrinology (1:30 PM-3:30 PM)

Title Both Estradiol and the Inhibins Are Critical for Restraint of FSH Secretion during the Follicular Phase in Normal Women

Author String CK Welt, KR Hayden-Karp, ND Shaw, S Srouji, JE Hall
Massachusetts General Hospital, Boston, MA

Body Context: Both estradiol (E2) and the inhibins suppress FSH but their roles in dynamic FSH changes across the menstrual cycle are less clear. Based on previous studies,¹ we hypothesized that E2 and the inhibins contribute differentially to the negative feedback control of FSH in the early (EFP) and late follicular phase (LFP).
Methods: Daily blood samples were drawn across two menstrual cycles. In the 2nd cycle, letrozole 20 mg was administered on two consecutive days starting on day 3 in the EFP (n=6) or when follicle size reached at least 16 mm in the LFP (n=9). FSH, E2, and inhibins A&B were measured. Hormone levels were compared before and after letrozole administration and between control and treatment cycles.
Results: In the EFP, E2 reached its nadir 24 hr after the 1st dose of letrozole (39 ± 2 to 12 ± 1 pg/mL; $p<0.001$) and remained lower than in the control cycle for 3 days. Peak FSH occurred 60 hr after the 1st dose of letrozole and was higher than in the control cycle (21.8 ± 3.8 vs. 14.5 ± 2.0 IU/L; $p<0.001$), but did not reach postmenopausal levels (>40 IU/L). The rise in FSH was followed within 24 hr by a rise in inhibin B, to levels that were higher than during the control cycle (275.0 ± 51.6 vs 73.3 ± 6.6 pg/mL; $p<0.05$). FSH began to fall on the day E2 began to increase, but the fall did not correlate with the inhibin B increase.
In the LFP, E2 reached its nadir 36 hr after the first dose of letrozole (123 ± 18 to 21 ± 4 pg/mL; $p<0.05$) and remained lower than in the control cycle for 5 days. Peak FSH occurred 48 hr after the 1st dose of letrozole and was higher than in the control cycle (21.1 ± 1.9 vs. 11.0 ± 1.6 IU/L; $p<0.01$). Higher FSH levels in letrozole cycles were followed by higher inhibin B (days 3-6 from letrozole; $p<0.05$) and inhibin A levels (day 3 from letrozole; 91.8 ± 18.9 vs. 37.1 ± 8.1 pg/mL; $p<0.05$) compared to control cycles. Although FSH decreased with the continued elevation of inhibin A, it remained higher than in control subjects until E2 rose to control levels.
Conclusion: In response to aromatase inhibition, the increases in FSH, but its failure to reach postmenopausal levels in the EFP and LFP demonstrate the combined importance of E2 and the inhibins in restraining FSH secretion throughout the normal follicular phase. In the LFP, inhibins A and B provide only partial negative feedback on FSH further supporting the importance of the combined effects of E2 and the inhibins in the negative feedback on FSH in the preovulatory phase.

Welt CK et al., J Clin Endocrinol Metab 2003; 88:1766

Disclosures: JEH: Study Investigator, Novartis Pharmaceuticals. Nothing to Disclose: CKW, KRH-K, NDS, SS

Pub # P3-191

Session Information POSTER SESSION: CLINICAL - Female Reproductive Endocrinology (1:30 PM-3:30 PM)

Title Gonadotropin Responses to Estrogen Positive and Negative Feedback in African-American and Caucasian Women

Author String ND Shaw, KM Klingman, SN Histed, SS Srouji, JE Hall
Massachusetts General Hospital and Harvard Medical School, Boston, MA; Children's Hospital Boston, Boston, MA

Body Context: Previous studies indicate that gonadotropin levels and cycle characteristics are similar in African American women (AAW) and Caucasian women (CW) despite higher estradiol (E2) levels across the cycle in AAW. Gonadotropin responses to a controlled sex-steroid infusion were measured in AAW and CW to determine if similar gonadotropin levels in the two groups are due to decreased sensitivity to estrogen feedback in AAW. As changes in E2 from the early-mid and mid-late follicular phase do not differ in AAW and CW, an alternative hypothesis is that feedback is dependent on changes in E2 between cycle phases rather than on absolute levels.

Methods: Healthy premenopausal AAW (n=10) and CW (n=13) were admitted in the early follicular phase for a 5-day, graded E2 and progesterone (P) infusion previously shown to result in negative followed by positive feedback. LH, FSH, E2, and P were measured every 4h. The LH or FSH nadir was calculated as a 3-pt moving average; the peak was defined as the highest value. LH and FSH are expressed in IU/L of the 2nd Pituitary International Standard 80/552. Data were analyzed using 2-tailed t-tests with corrections made for multiple comparisons ($p < 0.025$ considered statistically significant).

Results: AAW and CW were of similar age (mean [range]; 28.1 [22-36] yr) and BMI (24.4 [19-32] kg/m²). E₂ and P levels were within physiologic follicular phase ranges and did not differ between the 2 groups ([48-96 h mean \pm SE] E₂: 288 \pm 14 pg/mL; P: 1.4 \pm 0.1 ng/mL). There was no difference in negative feedback on LH (nadir 3.8 \pm 0.4 v 5.4 \pm 0.9 IU/L; time of nadir 33.2 \pm 3.3 v 32.3 \pm 2.7 h) or FSH (nadir 3.1 \pm 0.4 v 3.1 \pm 0.3 IU/L; time of nadir 48.8 \pm 2.7 v 50.5 \pm 3.1 h) in AAW compared to CW. There was also no difference in the positive feedback response to identical sex-steroid levels in AAW and CW for LH (peak 80.3 \pm 13.3 v 73.1 \pm 11.6 IU/L; time of peak 80.4 \pm 4.3 v 86.5 \pm 3.1 h) or FSH (peak 13.4 \pm 1.4 v 10.2 \pm 1.0 IU/L; time of peak 82.2 \pm 4.0 v 97.2 \pm 4.9 h).

Conclusions: 1. The negative and positive feedback LH and FSH responses to a controlled sex-steroid infusion do not differ between AAW and CW; 2. These data suggest that the identical LH and FSH levels in the context of higher estrogen levels in normally cycling AAW cannot be explained by diminished hypothalamic-pituitary sensitivity to E₂; and 3. These studies further suggest that the magnitude and timing of negative and positive feedback depend on changes in E₂ rather than on absolute levels.

Sources of Research Support: NIH Grants R01 AG13241; M01 RR1066; 5T32 HD007396.

Nothing to Disclose: NDS, KMK, SNH, SSS, JEH

Pub # P3-192

Session Information POSTER SESSION: CLINICAL - Female Reproductive Endocrinology (1:30 PM-3:30 PM)

Title Decreases in Serum Inhibin Levels Are Associated with Body Composition Changes across the Menopause Transition

Author String NS Akel, EJ Atkinson, S Khosla, LJ Suva, D Gaddy
University of Arkansas for Medical Sciences, Little Rock, AR; Mayo Clinic, Rochester, MN

Body The menopause transition is characterized by several physiological changes leading to altered body composition and functional activity. We previously showed that decreases in serum Inhibin A (InhA) levels were significant predictors of increases in bone turnover markers leading to decreased bone mass across the menopause transition. Others have shown that menopausal fluctuations of estradiol, decreased Inhibin B (InhB), and increased FSH levels were associated with increased aches, joint pain and stiffness, and vasomotor symptoms. Decreased grip strength and body composition changes are also characteristics of the perimenopause, although the hormonal basis of these changes is undefined. Therefore, we first determined that the effects of Inhibins on adipocyte differentiation are suppressive in human mesenchymal stem cell cultures. We then tested the hypothesis that decreases in serum Inhibins across the menopause transition are associated with increased body fat (changes in body composition) and decreased grip strength, using a cross-sectional age-stratified population sample of 106 pre- and postmenopausal women (ages 20-85 yr), excluding women using oral contraceptives or hormone replacement therapy. Both serum InhA and InhB levels significantly correlated inversely with waist and hip girth, and waist/hip girth ratio in premenopausal women. InhB levels were also inversely correlated with total fat, weight, and BMI in premenopausal women. No significant correlations with these measurements in post-menopausal women were observed for either InhA or InhB. When women were stratified by decade of life, negative correlations of InhA were observed only during midlife (age 45-54 for waist girth and waist to hip girth ratio). Similarly, InhB levels were inversely correlated during midlife in women age 35-44 for waist girth, and in women age 45-54 for waist and hip girth, and BMI. Moreover, serum InhB levels demonstrated significant positive correlations for grip strength, but only in women age 35-44. Together, these findings are consistent with the idea that the decreases in Inhibins that signal the onset of the menopause prior to loss of estrogens are also involved in the menopausal increase in waist/hip girth and BMI, perhaps via derepression of adipocyte differentiation. Moreover, the positive correlation of InhB with grip strength in midlife suggests a novel regulatory role for Inhibins in regulating muscle strength in women across the menopause transition.

Sources of Research Support: NIH R01DK-54044 and R21DK-74024 to DG, and R01 AR-027065 and P01 AG004875 to SK.

Nothing to Disclose: NSA, EJA, SK, LJS, DG

Pub # P3-193

Session Information POSTER SESSION: CLINICAL - Female Reproductive Endocrinology (1:30 PM-3:30 PM)

Title Reduced Sleep Quality Is Associated with Higher Sleep-Related LH Pulse Frequency in Peripubertal Girls

Author String J Collins, M Abshire, J Beller, C Burt Solorzano, J Marshall, C McCartney
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Body LH (and by inference GnRH) pulse frequency can be influenced by sleep. LH pulse frequency in girls increases during sleep in early puberty and decreases during sleep in late puberty (during the late follicular phase). A study of pubertal children (7 boys, 3 girls, ages 9-16 y, Tanner 2-5) suggested that LH pulses tended to occur in non-REM sleep (1). However, how altered sleep quality/architecture affects sleep-related LH pulse frequency in peripubertal girls remains unclear.

To assess the relationship between sleep quality and sleep-related LH pulse frequency, we studied 23 adolescent girls (age 8-17 y; Tanner stage 1-5 [mean \pm SD, 3.8 \pm 1.6]; BMI-for-age percentile [BMI%] 82.2 \pm 24.7, free testosterone [T] 29.2 \pm 36.7 pmol/L). If postmenarcheal, study occurred on cycle day [ge] 8; all progesterone values were $<$ 1.1 ng/ml. LH pulse frequency was determined via every 10 min blood sampling and analyzed by Cluster 7. Sleep efficiency (time asleep divided by designated sleep period) and number of awakenings were determined with wrist actigraphy. The relationship between average LH interpulse interval and sleep quality (sleep efficiency, number of awakenings) was assessed using partial Spearman rank (non-parametric) correlation procedures, adjusting for Tanner stage, BMI%, and free T--variables that could plausibly influence LH pulse frequency, sleep architecture, or both.

By wrist actigraphy, time from first to last sleep was 7.59 \pm 0.95 h, sleep efficiency 0.87 \pm 1.0%, and number of awakenings 12.7 \pm 6.1. There was no simple correlation between LH pulse frequency and either sleep efficiency or number of awakenings ($p >$ 0.2 for both). However, when adjusted for Tanner stage, BMI%, and free testosterone, LH interpulse interval correlated positively with sleep efficiency ($r_s = 0.477$, $p <$ 0.0336) and negatively with number of awakenings ($r_s = -0.456$, $p <$ 0.0435).

These early data suggest that poor sleep quality is associated with higher sleep-related LH pulse frequency in peripubertal girls. This is consistent with findings in adult women during the early follicular phase, in whom LH pulses tend to follow brief wake episodes (2). Importantly, these data help confirm that sleep quality is a potential confounder when analyzing LH pulse frequency in peripubertal girls and that sleep monitoring is important in such studies.

(1) Boyar R et al., N Engl J Med 1972; 287:582

(2) Hall JE et al., J Clin Endocrinol Metab 2005; 90:2050

Sources of Research Support: F32 HD066855 (JC); R01 HD058671 (CM); Eunice Kennedy Shriver National Institute of Child Health and Human Development/National Institutes of Health through cooperative agreement U54 HD28934 as part of the Specialized Cooperative Centers Program in Reproduction and Infertility Research (JM, CBS, MA); General Clinical Research Center Grant M01 RR00847.

Nothing to Disclose: JC, MA, JB, CBS, JM, CM

Pub # P3-194

Session Information POSTER SESSION: CLINICAL - Female Reproductive Endocrinology (1:30 PM-3:30 PM)

Title Effects of Dehydroepiandrosterone (DHEA) Treatment on Sleep Architecture and on Testosterone and Estradiol Levels in Postmenopausal Women

Author String A Caufriez, R Leproult, M L'Hermite-Baleriaux, M Kerkhofs, G Copinschi
Université Libre de Bruxelles, Brussels, Belgium; University of Chicago, Chicago, IL

Body The present randomized, double-blind, placebo-controlled study aimed to analyze in postmenopausal women the effects of a 3-week dehydroepiandrosterone (DHEA) treatment on sleep architecture, and on DHEA sulfate (DHEAS), testosterone (T) and estradiol plasma levels. Eight healthy non-obese postmenopausal women, 48-74 y.o. (median 55.5), who did not take any medication for [ge] 2 months, participated in 2 studies separated from each other by [ge] 1 month. Each study included a 20-day period during which the subjects took a capsule of either 50 mg DHEA or placebo daily. On the last day, blood samples were drawn at 4-h intervals for 24 hours and overnight polysomnography was performed. Non parametric tests were used for statistical calculations. All group values are expressed as the median [range]. DHEA treatment was associated with significant increases in 24-h levels of DHEAS (236 [93-404] [micro]g/dl vs 39 [30-137] [micro]g/dl; P = 0.01), T (23 [15-41] ng/dl vs 11 [7-18] ng/dl; P = 0.01) and estradiol (8.13 [7.21-13.25] pg/l vs 5.47 [3.78-9.53] pg/l; P = 0.02). Under placebo, individual 24-h DHEAS levels tended to correlate positively with corresponding T (rs = 0.68, P = 0.07) and estradiol values (rs = 0.63, P = 0.09). In contrast, no relation was found under DHEA treatment (P [ge] 0.80). The magnitude of increases varied considerably among individuals for DHEAS (17 to 33 [micro]g/dl), T (-0.4 to 27 ng/dl) and estradiol (-0.75 to 5.87 pg/dl), and did not correlate with corresponding levels under placebo (P = 0.66). There was no relation between individual increments in DHEAS levels and corresponding T and estradiol increases (P = 0.90). Total duration of non-rapid eye movement (Non-REM) sleep (228 [138-391] min vs 215 [57-304] min; P = 0.01) and total slow-wave activity (SWA) (reflecting duration and intensity of slow-wave, i.e deep, sleep) over the first 4 hours of sleep (382 [90-772] mV2 vs 174 [57-832] mV2 (P = 0.05) were significantly higher under DHEA than under placebo. Individual increases in SWA under DHEA treatment relative to placebo correlated positively with 24-h T levels under DHEA (rs = 0.86, P = 0.02), but not with DHEAS and estradiol values (P [ge] 0.24). Other sleep variables were not significantly altered. These results indicate that in postmenopausal women, individual T and estradiol changes induced by a 3-week DHEA administration are highly variable and quite unpredictable. Sleep promoting effects of DHEA might possibly be mediated by testosterone.

Nothing to Disclose: AC, RL, ML-B, MK, GC

Pub # P3-195

Session Information POSTER SESSION: CLINICAL - Female Reproductive Endocrinology (1:30 PM-3:30 PM)

Title Circulating Free Testosterone Is Positively Associated with Metabolic Syndrome and Type 2 Diabetes in a Racially and Ethnically Diverse Group of Women: The Framingham OMNI Cohort

Author String R Ngermaneepongthong, K Choong, TG Trivison, A-H Nguyen, JM Murabito, S Bhasin, VS Ramachandran, AD Coviello
Boston University, Boston, MA

Body Minority women have a disproportionately elevated risk of obesity and type 2 diabetes (T2DM). Higher free testosterone (FT) and lower sex hormone binding globulin (SHBG) are associated with obesity and T2DM in white women. The relationship of circulating FT and SHBG and metabolic disorders in minority women remains poorly characterized.

Objective: To examine the cross-sectional relations between circulating total testosterone (TT), FT, and SHBG with body mass index (BMI), metabolic syndrome (MetS), and T2DM in minority women.

Methods: FT, TT, and SHBG were measured in 214 minority women (35% Hispanic, 20% Black, 30% Asian/Pacific Islander, 15% Other) in the Framingham Heart Study (FHS) OMNI 2 Generation cohort. TT was measured by LC-MS/MS (sensitivity 2 ng/dL), SHBG by immunofluorometric assay (Delfia), and FT was calculated. Multivariable linear and logistic regression were used to examine the relations between TT, FT, SHBG and BMI, MetS and T2DM.

Results: Mean age was 42 ± 14 years, mean BMI 26 ± 6 kg/m². Fifty-three (25%) women were using exogenous hormones (EH). Fifty-two (24%) women were post-menopausal, twelve (6%) were current smokers, and eleven (5%) had T2DM. Mean TT was 29.7 ± 14.9 ng/dl, FT 3.2 ± 1.9 pg/ml, and SHBG 95.9 ± 84.4 nmol/L. In multivariable linear models, after adjusting for age, smoking, T2DM and menopause, BMI was positively associated with FT (β 0.08 95%CI 0.03, 0.13, per 1 unit increase in BMI) and negatively associated with SHBG (β -4.60 95%CI -6.50, -2.71, per 1 unit increase in BMI). After adjusting for age, BMI, smoking, and menopause, T2DM was associated with higher FT than women without T2DM (β 1.41 95%CI 0.18, 2.64). Women with FT in the highest quartile were more likely to have MetS (odds ratio [OR] 4.66, 95% CI 1.01, 21.68) compared to women with FT in the lowest quartile after adjusting for age, BMI, and menopause. Women with SHBG levels in the lowest quartile were more likely to have MetS (OR 7.90 95% CI 1.52, 40.95) compared to women with SHBG in the highest quartile. In analyses excluding women using EH, the association between higher FT levels and MetS persisted. We were unable to assess T2DM risk due to the low number of cases. TT had no significant relation to BMI, MetS, and T2DM.

Conclusions: Higher circulating FT and lower SHBG levels are associated with higher BMI, MetS, and T2DM in a community-based sample of minority women. These findings are consistent with prior findings in the FHS white women.

Sources of Research Support: R01 HL094755-03.

Nothing to Disclose: RN, KC, TGT, A-HN, JMM, SB, VSR, ADC

Pub # P3-196

Session Information POSTER SESSION: CLINICAL - Female Reproductive Endocrinology (1:30 PM-3:30 PM)

Title Vitamin D Deficiency Is Common in Pregnancy, but Not Associated with Spontaneous Miscarriage

Author String M Grossmann, EJ Hamilton, C Francis, A Tint, K Kuswanto, M Lappas, K Sikaris, Z Lu, JD Zajac, M Permezel, S Tong
University of Melbourne, Heidelberg, Australia; University of Melbourne, Heidelberg, Australia; Melbourne Pathology, Collingwood, Australia

Body

Introduction: Low maternal 25-hydroxyvitamin D3 (Vit D) levels have been associated with a number of adverse outcomes for both mother and child. Since Vit D has been implicated in immune regulation, we hypothesised low levels increases risk of spontaneous miscarriage.

Aim: To examine whether spontaneous miscarriage is associated with low serum Vit D levels in early pregnancy.

Methods: We conducted a case-control study in Australian women, comparing serum Vit D levels in women who miscarriage to those who had at least two successful births. Controls were women presenting for their first prenatal visit. Cases and controls were matched to estimated gestation on day of sample collection. Serum Vit D levels were measured by Diasorin Liaison (Diasorin, North Ryde NSW, Australia).

Results: We recruited 127 women who miscarried (miscarriage at median 9 weeks' gestation, range 5-18 weeks), and 164 controls (gestation age at delivery 39 weeks, range 32-42). Vit D level was higher in women with miscarriage, mean 54.2 nmol/L* (range <10 - 101) compared to controls, 47.4 nmol/L (13 - 106), $p < 0.05$. Of the entire cohort of women, only 12% were Vit D sufficient (defined as Vit D >75 nmol/L). 37.3% had Vit D insufficiency (50-75 nmol/L), 39.4% mild (25-49 nmol/L) and 11.3% severe (<25 nmol/L) Vit D deficiency.

Conclusions: Vit D deficiency is not associated with spontaneous miscarriage. While 88% of pregnant women were insufficient or deficient, the optimal Vit D level to reduce the risk of pregnancy-associated complication remains unknown.

*to convert to ng/ml, divide by 2.5.

Nothing to Disclose: MG, EJH, CF, AT, KK, ML, KS, ZL, JDZ, MP, ST

Pub # P3-197

Session Information POSTER SESSION: CLINICAL - Female Reproductive Endocrinology (1:30 PM-3:30 PM)

Title Outcomes of Spontaneous and Assisted Pregnancies in Turner Syndrome: The NIH Experience

Author String TN Hadnott, HN Gould, AM Gharib, CA Bondy
National Institutes of Health, Bethesda, MD; Howard Hughes Medical Institute, Chevy Chase, MD; National Institutes of Health, Bethesda, MD

Body Turner syndrome (TS) affects approximately 1 in 2,500 live-born females and causes infertility in most affected individuals. Case reports of spontaneous pregnancies and pregnancies following use of assisted reproductive technologies (ART) suggest high fetal and maternal complication rates for women with TS. To survey outcomes of spontaneous and ART pregnancies for a large cohort of women with TS, we obtained menstrual and obstetric histories, lymphocytic karyotypes, and cardiovascular imaging on adult participants in the NIH Turner syndrome natural history protocol between 2001 and 2010. Among the 276 TS women in our sample, 5 (1.7%) women had spontaneous pregnancy and menarche and 5 (1.7%) had assisted pregnancies (1 had both spontaneous and ART pregnancies). All of the women who spontaneously conceived had 45,X karyotype in $\geq 90\%$ of analyzed cells. In total there were 14 live births following 13 pregnancies, with 10 (76.9%) of the 13 deliveries being completed via Cesarean section. Four (57.1%) of seven infants from ART pregnancies were classified as low birth weight (less than 2,500 grams). One of the 15 children had cerebral palsy, but the others were reportedly chromosomally and clinically normal. The only serious maternal complication occurred in an ART-related twin pregnancy which featured preeclampsia requiring preterm delivery. To assess the potential effects of pregnancy on aortic dilation in TS, we compared aortic diameters obtained by cardiac magnetic resonance in parous versus nulliparous women. The average duration since most recent delivery and cardiovascular evaluation in the NIH protocol was 11 ± 3.2 years (range, 2 - 34 years). Ascending aortic diameters, measured at five distinct sites, were not significantly different in parous versus nulliparous TS women. Likewise, body size-adjusted aortic diameters were not significantly different among the two groups. No catastrophic complications occurred in 13 pregnancies, and fetal outcomes were generally good in this survey. However, the small sample size did not allow for assessing effects of important variables such as aortic valve structure, type of pregnancy (spontaneous versus ART), or number of pregnancies on the aorta in women with TS. The potential for life-threatening complications such as aortic dissection and death still warrant comprehensive cardiovascular screening prior to conception, single embryo transfer, and caution regarding unintentional pregnancies in this population.

Sources of Research Support: National Institutes of Health, Division of Intramural Research; HHMI-NIH Research Scholar Program, Howard Hughes Medical Institute.

Nothing to Disclose: TNH, HNG, AMG, CAB

Pub # P3-198

Session Information POSTER SESSION: CLINICAL - Female Reproductive Endocrinology (1:30 PM-3:30 PM)

Title High Incidence of Cardiac and Metabolic Abnormalities in a Cohort of Patients with Turner Syndrome (TS)

Author String VER Parker, SF Syed, A Webb, J MacDougall, M Belham, HL Simpson
Addenbrooke's Hospital, Cambridge, UK; Addenbrooke's Hospital, Cambridge, UK; Addenbrooke's Hospital, Cambridge, UK

Body **Objectives:** A retrospective review of adult patients with Turner Syndrome attending a multidisciplinary clinic.
Methods: Case notes and electronic records were reviewed in 62 patients.
Results: Diagnosis was prenatal/at birth in 20%, 1-10 years in 29%, 11-20 years in 47% and after 21 years in one patient. Common presenting complaints were short stature 51% and primary amenorrhoea 27%. The overall median height was 1.48m (range 1.30-1.62m). 21% were above the 95th Turner height centile, 39% between the 75th and 95th centile, 34% between the 25th-75th, and 6% were under the 25th. GH increased height range by 10cm. 4 TS mosaic patients achieved spontaneous puberty and 2 patients had spontaneous pregnancies. All premenopausal patients were taking oestrogen replacement: sequential combined HRT 43%, OCP 30%, continuous combined HRT 17%, topical oestrogen 6%, ethinyloestradiol alone 4%. Mean uterine volume was 47.24cm³ ±16.17.
32.5% of patients had structural cardiac abnormalities: aortic root dilatation 10% (>2cm/m²), coarctation of the aorta 3%, pseudocoarctation 8%, bicuspid aortic valve 6.5%, anomalous pulmonary venous drainage 2%, persistent left sided SVC 2%, aberrant right subclavian artery 1%. All findings were substantiated by echocardiography with cardiac MRI in 81% of cases. 39% of patients were hypertensive.
Additional metabolic abnormalities demonstrated included: hypercholesterolaemia 80%, type 2 diabetes 11%, type 1 diabetes 4%, glucose intolerance 6%. 29% patients had a BMI >30, 22% of patients had hypothyroidism and 2% Graves' disease. Coeliac antibodies were present in 6.5% of patients. 32% had abnormal LFTs, 15% had hepatic steatosis. Patients on continuous combined HRT had higher LFTs than those on other oestrogen preparations (P<0.05). 24% had structural renal tract abnormalities: horseshoe kidney was the commonest 9.6%. BMD was available in 65%; osteopaenia 51%, osteoporosis 8%.
Discussion: These data demonstrate the multi-system nature of TS, and in particular the high incidence of structural cardiac anomalies and metabolic abnormalities. There has been an increased rate of detection of structural cardiac anomalies since a cardiologist with an interest in TS has joined the service. To date there have been no patients with aortic dissection and all pregnancies progressed without incident. It will be of interest to determine whether TS clinics result in a reduced rate of cardiovascular events in the future.

Nothing to Disclose: VERP, SFS, AW, JM, MB, HLS

Pub # P3-199

Session Information POSTER SESSION: CLINICAL - Female Reproductive Endocrinology (1:30 PM-3:30 PM)

Title The Association of Serum Prolactin Concentration and Biomarkers of Systemic Inflammation: A Cross-Sectional Analysis in a Population-Based Study

Author String N Friedrich, HJ Schneider, C Spielhagen, H Wallaschofski, M Nauck
Institute of Clinical Chemistry and Laboratory Medicine, Greifswald, Germany; Medizinische Klinik - Innenstadt, Munich, Germany

Body Background: Prolactin (PRL) is involved in immune regulation and may contribute to an atherogenic phenotype(1). Previous results on the association of PRL with markers of inflammation are conflicting and limited by small patient studies, whereas data from large population-based studies are missing (2-7). Therefore, the aim of the present study was to assess the association between serum concentration of PRL and high-sensitive C-reactive protein (hsCRP), fibrinogen, interleukin-6 (IL-6), and white blood cells (WBC). Methods: From the Study of Health in Pomerania (SHIP), a representative population-based study, a total of 3774 subjects were available for the present analyses. Results: After adjustment for age, sex, body-mass-index, total cholesterol and glucose we detected a positive association of PRL with WBC in linear regression analyses. In logistic regression analyses, we found a significant association of PRL with increased IL-6 in non-smokers [highest vs. lowest quintile: odds ratio 1.65 (95% confidence interval 1.10-2.58), p=0.02] and smokers [OR 2.06 (95%-CI 1.10-3.89), p=0.02]. Similar results were found for WBC in non-smokers [highest vs. lowest quintile: OR 2.09 (95%-CI 1.21-3.61), p=0.01] but not in smokers. Linear and logistic regression analyses revealed no significant associations of PRL with hsCRP or fibrinogen. Conclusions: Serum PRL concentration is associated markers of inflammation such as IL-6 and WBC but not hsCRP or fibrinogen in the normal population. IL-6 additionally acts as an adipokine. This supports a role of PRL in inflammation and regulation of adipose tissue.

- (1) Ben-Jonathan N et al., *Endocr Rev* 2008; 29:1-41
- (2) Bahceci M et al., *Horm Res* 2004; 62:283-287
- (3) Tuzcu A et al., *Endocr J* 2005; 52:89-94
- (4) Serri O et al., *Clin Endocrinol (Oxf)* 2006; 64:366-370
- (5) Yavuz D et al., *Eur J Endocrinol* 2003; 149:187-193
- (6) Brismar K et al., *Gend Med* 2009; 6 Suppl 1:123-136
- (7) Balci H et al., *Pituitary* 2009; 12:170-176

Nothing to Disclose: NF, HJS, CS, HW, MN

Pub # P3-200

Session Information POSTER SESSION: CLINICAL - Female Reproductive Endocrinology (1:30 PM-3:30 PM)

Title Urinary Cortisol Metabolism in Women with Endometriosis

Author String O Muneyyirci-Delale, C Charles, J Anopa, M Dalloul, F Dailey, VL Nacharaju
SUNY Downstate Medical Center, Brooklyn, NY; Kings County Hospital Center, Brooklyn, NY

Body

Background and Objective: Endometriosis is a common gynecological condition that is frequently associated with chronic pelvic pain, infertility and increased stress. Stress has been associated with higher level of glucocorticoids, which have been shown to be involved in female reproductive function especially in the endometrium. However, there exists a link between high-stress conditions and hypocortisolism, which is suggestive of a disorder in the metabolism of cortisol that needs to be further studied. In this study, urinary cortisol metabolites were measured in premenopausal women with symptomatic endometriosis and healthy controls to assess changes in cortisol metabolism.

Material and Methods: IRB-approved consent was obtained by all subjects prior to enrollment. Spot urine samples were collected from 21 normal cycling women without menopausal symptoms: 11 controls and 10 women with surgically-diagnosed endometriosis and chronic pelvic pain then kept frozen at -80 [deg]. A segmental gas chromatography/mass spectrometry/mass spectrometry (GC/MS/MS) method was developed to quantize tetrahydrocortisol (THF), allo-tetrahydrocortisol (allo-THF) and free cortisol in first injection then tetrahydrocortisone (THE) and free cortisone in the subsequent injection. The ratios of tetrahydrocortisol (THF + allo-THF) to THE and allo-THF to THF were calculated to assess cortisol metabolism. Statistical analysis was done using student's T-test and Pearson's correlations.

Results: The ratio of allo-THF to THF in control and endometriosis group is (mean \pm SEM) 2.32 ± 0.85 and 2.69 ± 0.75 , respectively. There was no statistically significant difference in this ratios between the groups ($p=0.749$). Similarly, the ratios of (THF + allo-THF) to THE was not significantly different (mean \pm SEM for control: 0.90 ± 0.15 vs. endometriosis: 0.88 ± 0.15 ; $p=0.952$). No correlations were observed between the ratios and weight, height and BMI of these women.

Conclusion: In our preliminary studies, we found cortisol metabolism was not different in women with symptomatic endometriosis when compared to normal controls. Due to our small sample size, we were not able to adequately evaluate cortisol metabolism. However, urinary cortisol analysis remains a viable assessment technique.

Nothing to Disclose: OM-D, CC, JA, MD, FD, VLN

Pub # P3-201

Session Information POSTER SESSION: CLINICAL - Female Reproductive Endocrinology (1:30 PM-3:30 PM)

Title Multi-Hormonal Bio-Identical Replacement Therapy Map (MHRT) in Menopause: Significant Associations as a Function of Somatic and Neurological Symptom Clusters

Author String ER Braverman, K Perrine, UJ Damle, S Savarimuthu, S Yeldandi, M Kerner, K Dushaj, E Mo, P Reddy, P Reddy, M Li, D Stratton, K Blum
Weill Cornell College of Medicine, New York, NY; PATH Foundation NY, New York, NY; University of Florida College of Medicine & McKnight Brain Institute, Gainesville, FL

Body During menopause, women experience changes in hormones which have individual and inter-related functions. 74 females (mean age=60.23,[43,87]) completed a medical evaluation with hormone screening (leptin, DHEA sulfate, estradiol, estrone, FSH, LH, pregnenolone, progesterone, free and total testosterone (F and T Test), and TSH). A Menopause Questionnaire (MQ) assessed severity (1=never, 5=always) of 64 menopausal symptoms in 12 symptom domains: Neurological, Neuropsychiatric, Neuropsychological, Endocrine (Endo), Pulmonary (Pul), Musculoskeletal (Musc), Gastrointestinal (GI), Cardiovascular (CV), Dermatological (Derm), Genitourinary (GU), Immune (Imm), and Gynecological (Gyn). Only 13 patients completed follow up MQ's. Significant hormone/domain correlations of 120 performed were: Leptin/CV ($r=.41, p<.015$), DHEA/GU ($r=.30, p<.05$); FSH/Pul ($r=-.29, p<.05$); Pregnenolone/GU ($r=.40, p<.006$), /Imm ($r=.38, p<.008$); Test/total symptoms ($r=-0.34, p<.016$); TSH/Pul ($r=-.33, p<.03$) /Gyn ($r=.30, p<.05$); Estrone/Musc ($r=-0.43, p<.012$). In follow-up, FSH/Neuropsychiatric ($r=.56, p<.05$) and /Musc ($r=.67, p<.013$); DHEA/Imm ($r=.64, p<.04$); LH/Musc ($r=.59, p<.34$); F Test/Neuropsychiatric ($r=.64, p<.019$), /Musc ($r=.68, p<.01$), and /Derm ($r=.57, p<.04$); T Test/Imm ($r=.63, p<.028$); TSH/Endo ($r=-.62, p<.031$). An MQ factor analysis yielded 2 factors (1: Pul, GI, CV, Imm; 2: Musc, Gyn, the 3 Neurological domains). Only Factor 1 had significant correlations: 1/pregnenolone ($r=.37, p<.019$), /Leptin ($r=.38, p<.037$). Hormone levels factor analysis yielded 4 factors (1: DHEA, F and T test; 2: Estradiol, FSH, LH; 3: Estrone, Progesterone; 4: Leptin, Pregnenolone). The orthogonal factors and symptom domains, factor scores, quantity, and mean rating correlated significantly as follows: 1/GI ($r=.50, p<.018$), /Imm ($r=.52, p<.016$); 2/Endo ($r=-.45, p<.038$); 3/Musc ($r=-.46, p<.034$), /Derm ($r=-.49, p<.022$). Hormone factors and symptom domain correlations at follow-up were significant for 1/neurophysiological ($r=.85, p<.017$) and 4/GU ($r=.80, p<.031$). Stepwise regression analysis was significant only for symptom domain Factor 1 ($p<.004$) with hormone level predictors of TSH ($p<.009$) and Leptin ($p<.019$). Our findings show a complex web of hormone clusters contributes to multi-system symptomatology in menopause and highlights the need for menopause to be evaluated from a multi-hormonal perspective. We propose a paradigm shift where MHRT replaces the old nomenclature of HRT and await confirmation in a larger population.

Disclosures: KB: Chairman of the Board and Chief Scientific Officer, LifeGen Inc, Reward Deficiency Solutions Inc. Nothing to Disclose: ERB, KP, UJD, SS, SY, MK, KD, EM, PR, PR, ML, DS

Pub # P3-202
Session Information POSTER SESSION: CLINICAL - Female Reproductive Endocrinology (1:30 PM-3:30 PM)
Title 2011 -- The Gender Gap Continues
Author String K Amrein, T Pieber, A Fahrleitner-Pammer, A Amegah-Sakotnik, T Urbanic, JK Mader, D Wagner, I Zollner Schwetz, C Putz-Bankuti, A Langmann
Medical University of Graz, Graz, Austria; Medical University of Graz, Graz, Austria; Medical University of Graz, Graz, Austria; Medical University of Graz, Graz, Austria; Medical University of Graz, Graz, Austria

Body

Introduction

Male scientists are more successful than females in biomedical research. This difference is thought to be primarily caused by women's low occupation in the higher academic posts and lower integration of women in the scientific community, such as influential positions in scientific associations or editorial boards of journals [1, 2]. Although the proportion of female authors has increased in the last decades, women still contribute less to top rank medical journals [3].

Methods

We evaluated the first and senior authors' first name in a total of 40 top 10 ranked journals of 8 different medical categories over a 12-month period. In cases when only initials were provided or the gender could not be assigned, the author's gender was classified as [ldquo]unknown[rldquo]. Based on 2009 *ISI Web of Knowledge Journal Citation Reports* categories, we analysed the following medical specialties: *Endocrinology and Metabolism*, *General Internal Medicine*, *Critical Care Medicine*, *Anesthesiology*, *Surgery*, *Emergency Medicine*, *Radiology and Clinical Neurology*.

Results

In all evaluated medical categories, the proportion of medical research authored by women was significantly lower than those authored by men, with a much lower percentage of senior authors. 33% of all first authors were female, compared to 18% of all senior authors. In *Endocrinology and Metabolism*, we found 39% female first and 22% female senior authors. There were significant differences between the evaluated categories, with the lowest percentage of female authors (first and last) in the category *Surgery* (21 and 13), followed by *Emergency Medicine* (22 and 15) and *Critical Care Medicine* (26 and 14). Female researchers were better represented in *General Internal Medicine* (30 and 20%), *Radiology* (35 and 17), *Clinical Neurology* (39 and 17) and *Anesthesiology* (39 and 24).

Discussion

We found a wide variation between the medical specialties analysed, probably reflecting the varying percentage of female physicians and researchers, with the lowest proportion of female physicians in Orthopedic Surgery (4%) and the highest in General Pediatrics (52%; AMA 2006). Further research should investigate the underlying causes contributing to this disparity and assess possible strategies to encourage women in their career development and scholarly productivity.

- (1) Puuska HM. et al., *Scientometrics* 2010
- (2) Dickersin K. et al., *JAMA* 1998
- (3) Jagsi R. et al., *NEJM* 2006

Nothing to Disclose: KA, TP, AF-P, AA-S, TU, JKM, DW, IZ-S, CP-B, AL

Pub # P3-203

Session Information POSTER SESSION: CLINICAL - Female Reproductive Endocrinology (1:30 PM-3:30 PM)

Title Ethical Breaches in Reproductive Medicine: The Case of Dr. Michael Kamrava

Author String MS Rosenthal
University of Kentucky, Lexington, KY

Body In 2009, The Medical Board of California launched an investigation into the fertility practice of Michael Kamrava, M.D., after it became known that his patient, Nadya Suleman, gave birth to the first surviving set of octuplets on January 26, 2009. As a result of its investigation, the Medical Board of California began hearings to revoke Dr. Kamrava's medical license, based on the Suleman case, and two other cases involving high-number embryo transfer resulting in high order multiple pregnancies in women over 40. In October, 2010, it was revealed that the octuplet outcome in the Suleman case was consequent to IVF and the transfer of 12 embryos. Additionally, the Suleman case raised social, ethical and legal questions about suitable IVF candidacy since the octuplets were born to an American single mother of six children, on public assistance, who used a sperm donor. In the Fall of 2010 it was revealed, through testimony in the Kamrava licensing hearings, that Nadya Suleman has 29 additional frozen embryos in storage, which she could potentially request to have implanted. Dr. Kamrava's testimony reveals ethical and professional violations that call into question whether his practices were part of an experimental protocol on human subjects without their consent. Additionally, an original article by Dr. Kamrava in the International Journal of Fertility and Sterility (2010; 4-29-34) raises disturbing questions about his unregulated research, Belmont Report violations, and the exploitation of human subjects.

This presentation addresses three areas of ethical breaches in the Kamrava Case: clinical ethics breaches, professional ethics breaches, and research ethics breaches. It frames the discussion within the context of unregulated IVF; an absence of professional self-regulation; publication ethics; and socially produced infertility that embraces experimental methods as [ldquo]innovative therapy[rdquo].

Nothing to Disclose: MSR

Pub # P3-204

Session Information POSTER SESSION: CLINICAL - Male Reproductive Endocrinology II & Case Reports (1:30 PM-3:30 PM)

Title Hypogonadism with Estrogen Removal (HER): Effects of Androgens and Estrogens on Body Composition in Young Adult Men

Author String JS Finkelstein, S-AM Burnett-Bowie, BZ Leder, JC Pallais, EW Yu, BJ Thomas, ML Webb, KE Wulczyn, JM Youngner, H Lee
Massachusetts General Hospital, Boston, MA; Massachusetts General Hospital, Boston, MA; Massachusetts General Hospital, Boston, MA

Body Testosterone (T) therapy increases lean mass and decreases fat mass in men. Methods: To determine if these effects are due to T itself or to its conversion to estrogen (E), we recruited 2 cohorts of healthy men aged 20-50. All men received goserelin acetate (Zoladex[reg], AstraZeneca LP, 3.6 mg q4wk) to suppress endogenous T and E. Men in Cohort 1 (T/E+, n=198) were randomized to treatment with 1 of 5 doses of a T gel (AndroGel[reg], Abbott) daily for 16 weeks (G1-placebo; G2-1.25g; G3-2.5g; G4-5g; G5-10g). Men in Cohort 2 (T/E-, n=200) were randomized to the same T doses plus all men received anastrozole (Arimidex [reg], AstraZeneca LP, 1 mg/d) to block conversion of T to E. Total body fat and lean mass were assessed by DXA and subcutaneous (SC) fat and thigh muscle areas were measured by CT at wk 0 and 16. Changes were assessed within the T/E- cohort to assess T effects and between cohorts to assess E effects. If T has an independent effect on body composition, differences vs the placebo T group should most likely occur as the T dose increases within the T/E- cohort because E levels should be similarly low in all groups while T levels become progressively more discordant. Conversely, if E has an independent effect on body composition, differences between the T/E+ and T/E- cohorts should be observed in Groups 2-5 but not G1 because E levels should be similarly low in subjects in G1 of both cohorts but higher in G2-5 in the T/E+ cohort. Results: Mean serum T levels in G1-5 were 44, 187, 332, 538, and 829 ng/dL in the T/E+ cohort and 41, 186, 339, 435, and 786 ng/dL in the T/E- cohort (P=NS at each dose between cohorts). Changes in fat measures were similar across groups within the T/E- cohort. In men who received placebo T (G1), aromatase blockade had no effect on total body fat (P=0.36) or SC fat area (P=0.66). However, in men receiving T gel (G2-5), total body fat (P<0.001) and SC fat area (P<0.001) increased more in the T/E- than the T/E+ cohort. Lean mass and thigh muscle area decreased significantly in G1 vs G2-5 in the T/E- cohort (P<0.01 for all comparisons). Aromatase blockade had no effect on changes in lean mass (P=0.17 for G1, P=0.39 for G2-5) or thigh muscle area (P=0.65 for G1, P=0.36 for G2-5). Conclusions: These results suggest that T alone regulates lean mass and muscle size while E, but not T, regulates fat mass in men. Treatment of hypogonadal men with non-aromatizable androgens may have undesirable effects on fat accumulation.

Sources of Research Support: NIH Grants R01 AG030545, K24 KD02759, and RR-1066 and an investigator-initiated grant from Abbott. AndroGel[reg] was provided at no cost by Abbott; Zoladex[reg] and Arimidex [reg] were provided at no cost by AstraZeneca Pharmaceuticals LP.

Disclosures: JSF: Principal Investigator, Astra Zeneca; Solvay Pharmaceuticals, Inc. BZL: Consultant, Amgen; Principal Investigator, Amgen; Lilly USA, LLC. Nothing to Disclose: S-AMB-B, JCP, EWY, BJT, MLW, KEW, JMY, HL

Pub # P3-205

Session Information POSTER SESSION: CLINICAL - Male Reproductive Endocrinology II & Case Reports (1:30 PM-3:30 PM)

Title Hypogonadism with Estrogen Removal (HER): Effects of Androgens and Estrogens on Lipid Profiles in Young Adult Men

Author String S-AM Burnett-Bowie, AF Moore (deceased), BZ Leder, HS Lee, EW Yu, LF Borges, BF Jones, AH Linker, JS Finkelstein
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Body Studies examining the effects of androgens and estrogens on lipids in men have produced inconsistent results. Methods: To assess the effects of androgens and estrogens on lipids in men, we recruited 2 cohorts of healthy men aged 20-50. All men received goserelin acetate (Zoladex[reg], AstraZeneca LP, 3.6 mg q4wk) to suppress endogenous testosterone (T) and estradiol (E). Men in Cohort 1 (T/E+, n=198) were randomized to treatment with 1 of 5 doses of a T gel (AndroGel[reg], Abbott) daily for 16 weeks (G1-placebo; G2-1.25g; G3-2.5g; G4-5g; G5-10g). Men in Cohort 2 (T/E-, n=200) were randomized to the same T doses plus all men received anastrozole (Arimidex[reg], AstraZeneca LP, 1 mg/d) to block conversion of T to E. Fasting total cholesterol, HDL, LDL, and triglycerides were measured every 4 weeks. Changes were assessed within the T/E- cohort to assess T effects and between cohorts to assess E effects. If T has an independent effect on lipids, differences vs the placebo T group should most likely occur as the T dose increases within the T/E- cohort because E levels should be low in all groups while T levels become more discordant. Conversely, if E has an independent effect on lipids, differences between the T/E+ and T/E- cohorts should be observed in Groups 2-5 but not G1 because E levels should be similarly low in subjects in G1 of both cohorts but higher in G2-5 in the T/E+ cohort. Results: Mean serum T levels in G1-5 were 44, 187, 332, 538, and 829 ng/dL in the T/E+ cohort and 41, 186, 339, 435, and 786 ng/dL in the T/E- cohort (P=NS at each dose between cohorts). Changes in LDL were similar in G1 vs G2-5 in both cohorts. Aromatase blockade had no effect on changes in LDL (P=0.48 for G1, P=0.37 for G2-5). In both cohorts, HDL levels increased more in men who received placebo T (G1) than in men who received T (G2-5) (P<0.03 vs G3, 4, and 5 of T/E+ and P<0.005 vs each group of T/E-). Aromatase blockade had no effect on the rise in HDL in men receiving placebo T gel (G1, P=0.69). However, in men receiving T gel (G2-5), HDL increased less (P<0.01) in the T/E- than the T/E+ cohort. Conclusions: Across a wide range of levels, androgens and estrogens have no effect on LDL levels in men. In contrast, T withdrawal increases HDL levels in men and aromatase blockade attenuates these increases. These results suggest that using non-aromatizable androgens to treat hypogonadal men may have undesirable effects on HDL levels in adult men.

Sources of Research Support: NIH Grants R01 AG030545, K24 KD02759, and RR-1066 and an investigator-initiated grant from Abbott. AndroGel[reg] was provided at no cost by Abbott; Zoladex[reg] and Arimidex [reg] were provided at no cost by AstraZeneca Pharmaceuticals LP.

Disclosures: BZL: Consultant, Amgen; Principal Investigator, Amgen; Lilly USA, LLC. JSF: Principal Investigator, Astra Zeneca; Solvay Pharmaceuticals, Inc. Nothing to Disclose: S-AMB-B, AFM, HSL, EWY, LFB, BFJ, AHL

Pub # P3-206
Session Information POSTER SESSION: CLINICAL - Male Reproductive Endocrinology II & Case Reports (1:30 PM-3:30 PM)
Title Hypogonadism with Estrogen Removal (HER): Effects of Androgens and Estrogens on Sexual Desire in Young Adult Men

Author String BZ Leder, H Lee, EW Yu, S-AM Burnett-Bowie, JC Pallais, ML Webb, KE Wulczyn, AB Servais, JS Finkelstein
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Body Testosterone (T) is thought to be the primary hormonal regulator of libido/sexual desire (SD) in men. Methods: To determine if SD is also regulated by estrogen (E), we recruited 2 cohorts of healthy men aged 20-50. All men received goserelin acetate (Zoladex[reg], AstraZeneca LP, 3.6 mg q4wk) to suppress endogenous T and E. Men in Cohort 1 (T/E+, n=198) were randomized to treatment with 1 of 5 doses of a T gel (AndroGel[reg], Abbott) daily for 16 weeks (G1-placebo; G2-1.25g; G3-2.5g; G4-5g; G5-10g). Men in Cohort 2 (T/E-, n=200) were randomized to the same T doses plus all men received anastrozole (Arimidex [reg], AstraZeneca LP, 1 mg/d) to block conversion of T to E. SD was assessed using both the International Index of Erectile Function (SD-M1) and a previously-validated question asking subjects to compare their sex drive now with baseline levels (-2=much less, -1=somewhat less, 0=the same, +1=somewhat more, +2=much more) (SD-M2). Changes were assessed within the T/E- cohort to assess T effects and between cohorts to assess E effects. Specifically, if T has an independent effect on SD, subjects in the T/E- cohort who receive no or little testosterone replacement should experience decreased SD compared to those receiving higher doses (as E levels will be the same). Conversely, if E has an independent effect on SD, differences between the T/E- and T/E+ cohorts should be observed in Groups 2-5 but not G1 (because E levels should be similarly low in subjects in G1 of both cohorts but higher in G2-5 in the T/E+ cohort). Results: Mean serum T levels in G1-5 were 43, 173, 346, 477, and 882 ng/dL in the T/E+ cohort and 34, 199, 329, 475, and 857 ng/dL in the T/E- cohort (P=NS at each dose between cohorts). SD declined more in men receiving placebo T than in the other T dose groups in both the T/E+ (P<0.005 vs G2, 3, 4 and 5 by SD-M1 and SD-M2) and the T/E- (P<0.02 vs G4 and G5 by SD-M1 and P<0.01 vs G3, 4, and 5 by SD-M2) cohorts. Aromatase inhibition further reduced SD in men receiving T gel (G2-5, P<0.0001 by both SD-M1 and SD-M2) but had no effect on SD in men receiving placebo T gel (G1, P=0.72 by SD-M1 and P=0.53 by SD-M2). Conclusions: As expected, lowering T levels reduces SD in men. Surprisingly, SD is further reduced in men treated with an aromatase inhibitor to reduce E production. These results suggest that non-aromatizable androgens may be less effective than aromatizable androgens for hypogonadal men with low sexual desire.

Sources of Research Support: NIH Grants R01 AG030545, K24 KD02759, and RR-1066 and an investigator-initiated grant from Abbott. AndroGel[reg] was provided at no cost by Abbott; Zoladex[reg] and Arimidex [reg] were provided at no cost by AstraZeneca Pharmaceuticals LP.

Disclosures: BZL: Consultant, Amgen; Principal Investigator, Amgen; Lilly USA, LLC. JSF: Principal Investigator, Astra Zeneca; Solvay Pharmaceuticals, Inc. Nothing to Disclose: HL, EWY, S-AMB-B, JCP, MLW, KEW, ABS

Pub # P3-207

Session Information POSTER SESSION: CLINICAL - Male Reproductive Endocrinology II & Case Reports (1:30 PM-3:30 PM)

Title Safety and Tolerability of LGD-4033, a Novel Non-Steroidal Oral Selective Androgen Receptor Modulator (SARM), in Healthy Men

Author String S Basaria, L Collins, M Sheffield-Moore, EL Dillon, K Orwoll, KM Lakshman, R Miciek, T Storer, J Ullloor, A Zhang, S Bhasin
Boston University School of Medicine, Boston, MA; University of Texas Medical Branch, Galveston, TX

Body **Background:** Sarcopenia is a common consequence of aging and chronic illness. Testosterone administration increases muscle mass and strength, but concerns about potential adverse effects on the prostate have restrained enthusiasm for its use as anabolic therapy. Selective Androgen Receptor Modulators (SARMs) are a class of androgen receptor (AR) ligands that display tissue-selective activation of androgenic signaling. LGD-4033 is a novel non-steroidal, orally active SARM that binds to AR with a K_i of ~ 1 nM and high selectivity ($\sim 4,000$ -fold) compared to other nuclear receptors. In animal models, LGD-4033 demonstrated an anti-resorptive and anabolic activity in muscles and bones, and high degree of selectivity for muscle versus prostate. In single ascending dose study, the safety and tolerability of LGD-4033 has been established up to 2.0 mg. In this Phase-1 study, we evaluated the safety and tolerability of multiple ascending doses of LGD-4033 administered daily for 20 days in healthy men.

Methods: In this placebo-controlled, Phase-1 multiple ascending dose study, healthy men (age 21-50) were randomized to 0.1, 0.3, or 1 mg LGD-4033 or placebo daily for 20 days. Liver function tests, fasting lipids, blood counts, PSA and ECG were monitored throughout the treatment period and the subsequent 5 weeks of observation phase. As an exploratory objective, body composition was measured using DEXA scan and maximal voluntary strength was measured using 1-RM method.

Results: LGD-4033 was well tolerated. There were no drug-related serious adverse event and none of the subjects was discontinued due to adverse events. All adverse events were mild to moderate in nature with no statistical difference between the active and placebo groups. There were no significant changes in hemoglobin, hematocrit or serum PSA at any dose. QT-interval did not change significantly during the intervention period. There was no indication of hepatotoxicity. Dose-dependent reduction in HDL cholesterol was seen in the LGD-4033 groups. Total lean body mass increased while fat mass decreased in the LGD-4033 groups. No changes in maximal voluntary strength were seen over the short treatment period.

Conclusion: In healthy young men, short-term treatment with LGD-4033 was safe and well tolerated. Longer intervention studies are needed to evaluate the efficacy of this non-steroidal SARM on body composition, muscle strength, and physical function.

Sources of Research Support: Ligand Pharmaceuticals, La Jolla, CA.

Nothing to Disclose: SB, LC, MS-M, ELD, KO, KML, RM, TS, JU, AZ, SB

Pub # P3-208
Session Information POSTER SESSION: CLINICAL - Male Reproductive Endocrinology II & Case Reports (1:30 PM-3:30 PM)
Title Effects of Testosterone and rhGH on Metabolic Syndrome Components in Older Men: The HORMA Study
Author String J He, S Bhasin, E Binder, C Castaneda-Sceppa, K Yarasheski, ET Schroeder, SP Azen, R Roubenoff, FR Sattler
Los Angeles, Los Angeles, CA; Boston University, Boston, MA; Washington University, St Louis, MO; Northeastern University, Boston, MA; University of Southern California, Los Angeles, CA; University of Southern California, Los Angeles, CA; University of Southern California, Los Angeles, CA; Tufts University Boston, MA

Body **Background:** Older persons are at risk for complications of the Metabolic Syndrome (MS) including heart attack, stroke, and vascular disease. **Methods:** Men 65-90 years-old (n=112) with testosterone (T) <550ng/dL and IGF-1 in lower adult tertile were randomly assigned to receive transdermal T (5 vs 10g/day) and rhGH (0, 3, or 5[μg/kg/day])(1). Lean body mass (LBM) and fat mass were measured by DEXA, T levels by mass spectrometry, and insulin, IGF-1, and adiponectin by chemiluminescence (automated immulite analyzer). MS parameters were scored as -1 if favorable and +1 if adverse predetermined thresholds were achieved; values not reaching these thresholds were assigned 0. MS parameters were related to changes in T and IGF-1 levels and body composition using pathway analysis. **Results:** After 16 weeks of treatment, the following parameters changed significantly (p<0.05): T (+320±478ng/dL), IGF-1 (+58±59ng/mL), total LBM (+1.79±1.89kg), total fat mass (-1.33±1.74kg), trunk fat (-0.88±1.21kg)*, BMI (+0.16±0.64), systolic blood pressure (+12.4±14.4mmHg)*, fasting triglycerides (-18.2±57.0mg/dL)*, HDL cholesterol (+3.46±6.65mg/dL)*, insulin sensitivity (QUICKI, -0.0011±0.0153)*, and HMW adiponectin (-0.18±0.76)*. MS scores (composite of MS elements designated by *) decreased -0.58±1.91 (p=0.002). In the pathway analysis, [uarr] in T and IGF-1 were significant (p<0.05) predictors of change in total fat and LBM, which were mediators (p<0.05) of change in BMI which was then a mediator (p<0.05) of change in MS parameters leading to change ([Delta]) in MS scores. The model fits the data well (overall chi-square=32.25, with 37 degrees of freedom, null p=0.65) as depicted by [Delta] in T and IGF-1[rarr][Delta] in LBM and total fat[rarr][Delta] in BMI[rarr][Delta] in MS parameters [rarr]improved MS scores. In the pathway, change in T and IGF-1 were only associated with changes in LBM and fat (except change in T independently affected adiponectin) which were not associated with change in MS components but latter were directly affected by change in BMI. None of the parameters explained increases in blood pressure. **Conclusions:** In older men, T supplementation with or without rhGH affects some components of the MS beneficially and others adversely but together improve MS composite scores. Change in BMI is the best predictor of change in MS elements and is jointly affected by both total fat and LBM.

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Sources of Research Support: National Institutes of Health R01 AG18169 and NCRR GCRCs M01 RR00004; at USC, M01 RR000054 at Tufts, and M01 RR000036 at Washington University. Study therapies (no monetary support) were provided by Solvay Pharmaceuticals Inc, Genentech Inc, and Tap Pharmaceutical Products Inc.

Disclosures: SB: Principal Investigator, Solvay Pharmaceuticals, Inc. EB: Principal Investigator, Solvay Pharmaceuticals, Inc. FRS: Principal Investigator, Solvay Pharmaceuticals, Inc. Nothing to Disclose: JH, CC-S, KY, ETS, SPA, RR

Pub # P3-209
Session Information POSTER SESSION: CLINICAL - Male Reproductive Endocrinology II & Case Reports (1:30 PM-3:30 PM)
Title Effects of Testosterone Treatment on Inflammatory Markers in Older Men
Author String M Maggio, G Ceda, Y Milaneschi, C Cattabiani, PJ Snyder, L Ferrucci
University of Parma, Parma, Italy; University Hospital, Parma, Italy; National Institutes of Health (NIH), Baltimore, MD; University of Pennsylvania School of Medicine, Philadelphia, PA

Body During aging in men there is a progressive reduction in testosterone levels and an increase in inflammatory markers (1-2). A causal relationship has been hypothesized (3-5) but never been tested in a randomized clinical trial.
Aim of the Study. To test the effects of transdermal testosterone on inflammatory markers.
Methods. 108 men [ge]65 years were selected by testosterone levels <1 SD below the mean for normal young men, 475 ng/dL, and were randomized to receive a testosterone or placebo patch in a double blind fashion for 36 months. Ninety-six subjects completed 36 months of treatment. The present study was performed in 70 men, 42 in the testosterone group and 28 in the placebo group who had sufficient sera available for assay. We measured serum concentrations of testosterone, c-reactive protein (CRP), interleukin-6 (IL-6), soluble interleukin-6 receptors (sIL6r and sgp130), soluble TNF-alpha receptor 1 (sTNFR1) by immunoassays. Body composition had been measured previously by DXA. Statistical analyses were performed using random-effect regression analyses, modelling an unstructured covariance matrix with slope and intercept as random effects. Results. The mean age at baseline was 71.8 ± 4.9 years in all 70 subjects. Testosterone- and placebo-treated groups had similar values for age, inflammatory markers and fat mass at baseline. The testosterone-treated group had lower levels of testosterone and BMI at baseline, but the differences were not statistically significant (p values of 0.06 and 0.07, respectively). Testosterone levels rose significantly following initiation of treatment in the testosterone-treated group but not in the placebo group. CRP levels were 1.58 ± 3.33 and 1.48 ± 2.75 at baseline in T and placebo-group, respectively. After 36 months of treatment, CRP levels increased in both the T (2.79 ± 4.10) and placebo (9.97 ± 24.77) groups. Similar trends were observed for the other inflammatory markers. A significant treatment*time interaction term, indicating a less steep increase in T-treated subjects compared to placebo-treated, was found only for CRP (p=0.03) and TNFR1 (p=0.02). No significant differences were found for IL-6 and soluble Interleukin-6 receptors (sIL6r and sgp130). Conclusion. Transdermal testosterone treatment of men [ge]65 years for 36 months was associated with less steep increase in CRP and TNFR1 than placebo treatment.

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Sources of Research Support: National Institute on Aging.

Nothing to Disclose: MM, GC, YM, CC, PJS, LF

Pub # P3-210

Session Information POSTER SESSION: CLINICAL - Male Reproductive Endocrinology II & Case Reports (1:30 PM-3:30 PM)

Title Is the Hematopoietic Effect of Testosterone Mediated by Erythropoietin? The Results of a Clinical Trial in Older Men

Author String M Maggio, G Ceda, Y Milaneschi, F Lauretani, C Cattabiani, PJ Snyder, L Ferrucci
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Body

In the elderly, low testosterone levels are predictors of anemia(1), which is consistent with the known stimulatory effect of testosterone on erythropoiesis(2). The mechanism(s) underlying the erythropoietic effect of testosterone are unknown.

Aim of the Study
To test the effects of transdermal testosterone on hemoglobin and erythropoietin levels in older men with low-normal levels of testosterone.

Methods
108 men >65 years old were selected by a serum testosterone concentration [ge]1 SD below the mean for normal young men (<475 ng/dL) and randomized to receive a testosterone or placebo patch in a double blind fashion for 36 months. Ninety-six subjects completed 36 months of treatment. The present analysis was performed on 70 men, 42 in the testosterone treatment group and 28 in placebo group who had sufficient sera remaining for assay of testosterone, hemoglobin, erythropoietin, and creatinine. Total testosterone was assessed by electrochemiluminescence immunoassay with minimum detective concentration (MDC) of 2 ng/d and interassay coefficients of variation (CV) <10%. Erythropoietin was assessed by ELISA with MDC of 2.5 mIU/ml and interassay CV <10%.

Statistical analyses were performed using random-effect regression, modelling an unstructured covariance matrix with slope and intercept as random effects. Random-effects models were valuable in this context, allowing the detection of variation between subjects and autocorrelation between repeated measurements of the same participants over time.

Results
The mean age \pm SD of the 70 subjects at baseline was 71.8 ± 4.9 years. The testosterone and placebo groups did not differ at baseline in age, hemoglobin, and erythropoietin. The testosterone-treated group had lower baseline testosterone and BMI than the placebo group but neither was quite statistically significant ($p=0.06$ and $p=0.07$, respectively). Testosterone replacement therapy for 36 months, as compared to placebo, induced significant increase in haemoglobin ($\beta \pm SE = 0.86 \pm 0.31$, $p = 0.01$) but no change in erythropoietin levels ($\beta \pm SE = -0.24 \pm 2.16$, $p=0.91$).

Conclusion
Transdermal testosterone treatment of men [ge] 65 years for 36 months significantly increased their hemoglobin levels but not their erythropoietin levels. Other mechanisms should be explored to explain the hematopoietic effect of testosterone.

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Sources of Research Support: National Institute on Aging.

Nothing to Disclose: MM, GC, YM, FL, CC, PJS, LF

Pub # P3-211
Session Information POSTER SESSION: CLINICAL - Male Reproductive Endocrinology II & Case Reports (1:30 PM-3:30 PM)
Title Long-Term Testosterone Administration Reduces Circulating Leptin Concentrations, without Significant Changes in Body Fat Measures, in Healthy Older Men

Author String H-W Lee, R Muniyappa, G Cizza, SM Harman, MR Blackman
National Institutes of Health, Bethesda, MD; National Institutes of Health, Bethesda, MD; National Institutes of Health, Baltimore, MD; Kronos Longevity Research Institute, Phoenix, AZ; Johns Hopkins University School of Medicine, Baltimore, MD; Veterans Affairs Medical Center, Washington, DC, Washington, DC

Body **Background:** Aging is associated with increased body fat, decreased circulating concentrations of testosterone (T) and increased leptin. A few reports suggest that T administration to hypogonadal younger men decreases adiposity and serum leptin. However, data regarding the effects of T supplementation in healthy older men are sparse.
Objective: We examined the baseline relationships among adiposity, bone mineral density (BMD), biomarkers of bone turnover, and serum leptin concentrations in 71 healthy older men (65-88 yr) with low to low-normal T levels (< 470 ng/dl). In a subgroup of these men (n = 35), we assessed the effects of low-dose T administration (100 mg IM every 2 weeks) for 26 weeks on serum leptin using a double-masked, placebo-controlled randomized study design (T group, n=16; placebo group, n= 19).
Methods: Adiposity was measured by body mass index (BMI), waist-to-hip ratio (WHR), DEXA (absolute and % total body fat, TBF), and abdominal MRI (total abdominal fat, TAF); BMD by DEXA (Femoral neck and distal radius); and bone turnover by serum concentrations of osteocalcin and procollagen peptide (PICP) and urinary excretion of deoxypyridinoline (DPD) crosslinks.
Results: Linear regression analyses at baseline revealed that serum leptin concentrations were directly related (P< 0.01) to measures of adiposity (BMI: r = 0.54; WHR: r = 0.35; TBF: r = 0.79; and TAF: r = 0.56). However, leptin was not significantly related to body fat-adjusted site-specific BMD or markers of bone turnover, nor to age, or serum concentrations of total T or free T. In a subgroup of these men, T supplementation did not significantly affect BMI, absolute or percent TBF or TAF, but decreased serum leptin concentrations by 29% vs. placebo (change from baseline: -29 % vs. -8.1 %, P= 0.01, adjusted for age, baseline serum leptin, and changes in total abdominal fat from baseline).
Conclusions: In healthy older men, total and abdominal fat predict baseline serum leptin, yet long-term T administration decreases serum leptin concentrations without changing body fat. These data suggest that T alters adipocyte leptin production, leptin metabolic clearance rate, or both.

Sources of Research Support: In part by the Intramural Research Programs of the National Center for Complementary and Alternative Medicine, Bethesda, Maryland and the National Institute on Aging, Baltimore, Maryland; National Institutes of Health Research Grant RO-1 AG11005; General Clinical Research Center Grant MO-1-RR-02719, from the National Center for Research Resources, National Institutes of Health, Bethesda, MD 20892; and the Research Service, Veterans Affairs Medical Center, Washington DC.

Nothing to Disclose: H-WL, RM, GC, SMH, MRB

Pub # P3-212
Session Information POSTER SESSION: CLINICAL - Male Reproductive Endocrinology II & Case Reports (1:30 PM-3:30 PM)
Title Polycythemia Is Common during Any Testosterone Replacement and Suppressed Gonadotropin May Predict Polycythemia in Primary Hypogonadism
Author String TT Agustsson, B McGowan, J Powrie, PV Carroll
Guy's and St Thomas' NHS Foundation Trust, London, UK

Body
Background
Testosterone replacement therapy results in a wide range of benefits for men with hypogonadism. Associated risks include polycythaemia. The aim of this study is to assess the prevalence of polycythaemia in men treated with different testosterone replacement therapies and to assess changes in associated biochemical markers with possible implications for patient management.

Methods

This is a retrospective observational study. We analysed biochemical and haematological parameters of all men on testosterone therapy who attended our endocrinology unit from the 1st of January 2009 until the 30th of June 2010. Of a total of 173 men, 86 (50%) were treated with testosterone undecyrate (Nebido^[reg]), 57 (33%) with transdermal testosterone gel, and 30 (17%) with intramuscular testosterone in the form of Sustanon^[reg]. Data were collected on haemoglobin concentrations, packed cell volumes, gonadotrophins, and total serum testosterone concentrations. Polycythaemia was defined as haemoglobin concentration >17 g/dl or packed cell volume >0.505.

Results

Out of the 173 men, 25 (14.5%) developed polycythaemia on at least one blood sample during the follow up period. 12 of the 86 men treated with Nebido^[reg] (14%), 8 out of the 57 men treated with transdermal gel (14%), and 5 of the 30 men treated with Sustanon^[reg] (17%) developed polycythaemia. There was no significant difference between the different treatment modalities.

Twenty eight percent of all men with polycythaemia had at least one elevated testosterone reading compared with 26% of men without polycythaemia. There was no significant difference in mean testosterone (16.1 mmol/L vs 16.9 mmol/L). 69.8% of men with primary hypogonadism and polycythaemia had suppressed gonadotrophins compared with 25% of men without polycythaemia.

Conclusions

We found no reliable correlation between testosterone levels and polycythaemia but suppressed gonadotrophin was associated with the development of polycythaemia in primary hypogonadism. Polycythaemia is a common risk with any testosterone treatment modality. Some previous studies have indicated that this is less likely with transdermal preparations, but we found the risk to be equal in all treatment groups.

These findings demonstrate the importance of careful monitoring of haematological variables during any testosterone treatment and suggest that suppressed gonadotrophin may have a greater predictive value than testosterone measurements in hypogonadism.

Nothing to Disclose: TTA, BM, JP, PVC

Pub # P3-213

Session Information POSTER SESSION: CLINICAL - Male Reproductive Endocrinology II & Case Reports (1:30 PM-3:30 PM)

Title Lower Plasma Testosterone Is Associated with Higher Framingham Risk Score

Author String B Chock, T-C Lin, C-S Li, A Swislocki
Veterans Affairs Northern California Health Care System (VANCHCS), Mather, CA; University of California, Davis, Sacramento, CA; University of California, Davis, Davis, CA; University of California, Davis, Davis, CA

Body

Background:
The Framingham risk score predicts a patient's 10 year risk of developing cardiovascular disease. Many risk factors included in the calculation of this score influence (or are influenced by) circulating testosterone.

Purpose:
To further clarify the possible association between testosterone and cardiovascular risk, as defined by the Framingham score, we analyzed data from a Veterans Affairs database.

Methods:
A retrospective chart review was performed. Inclusion criteria were male sex and age ≥ 20 . Exclusion criteria included pre-existing cardiovascular disease, stroke, and diabetes. Testosterone supplements, antiandrogen therapy, antihypertensives, or lipid-lowering medications were not exclusion criteria, but were factored in data analysis. Laboratory and clinical data were collected on veterans who had total plasma testosterone checked in the year 2008. Framingham scores were calculated using an on-line tool from the National Heart Lung and Blood Institute. Using SAS v9.2, robust linear regression assessed the association between total testosterone and Framingham score. The Spearman rank correlation coefficient, Wilcoxon rank-sum test, and Kruskal-Wallis test evaluated associations between total testosterone and the other patient data. A p-value ≤ 0.05 was considered significant.

Results:
1,479 patients were included in the study. Mean age was 61 years. There were significant negative associations between total testosterone and Framingham score (estimate of regression parameter for total testosterone -0.0042, $p < 0.0001$) and between free testosterone and Framingham score (estimate of regression parameter for free testosterone -0.2744, $p < 0.001$). Total testosterone was positively associated with HDL and negatively associated with BMI, total cholesterol, triglycerides, and number of blood pressure medications used. Free testosterone was positively associated with total cholesterol and LDL and negatively associated with age, BMI, and number of blood pressure medications used. There was no statistically significant association between BMI and Framingham score.

Conclusions:
Lower plasma testosterone may suggest the presence of other more traditional cardiovascular risk factors and potentially increased risk for heart disease.

Sources of Research Support: NIH Grant (National Center for Research Resources [NCRR] and NIH Roadmap for Medical Research) UL1 RR024146.

Nothing to Disclose: BC, T-CL, C-SL, AS

Pub # P3-214

Session Information POSTER SESSION: CLINICAL - Male Reproductive Endocrinology II & Case Reports (1:30 PM-3:30 PM)

Title Absence of a Relationship between Sex Hormones and Cognitive Performance in a Cross-Sectional Study of Men with Substance Use Disorders in an Inner-City Population

Author String MF Zilbermint, X Xu, A Wisniewski, O Selnes, AS Dobs
Johns Hopkins University School of Medicine, Baltimore, MD; Saint Agnes Hospital, Baltimore, MD; University of Oklahoma Health Sciences Center, Oklahoma City, OK; Johns Hopkins University School of Medicine, Baltimore, MD; Johns Hopkins University School of Medicine, Baltimore, MD

Body Hypogonadism is common with opiate-like drug use and might contribute to cognitive abnormalities. With the increasing epidemic of HIV and substance use disorders (SUD) world wide, it is important to understand the possible impact of these conditions on cognitive function, which may affect quality of life, and possibly decrease adherence to treatment. We hypothesized that men with SUD, by virtue of hypogonadism secondary to HIV and/or drug use, may exhibit impaired cognition.

We recruited men from the population of inner-city individuals living in Baltimore, Maryland. All were aged 18-50, who were active and non-active illicit drug users with positive and negative HIV status. Morning serum blood levels of total testosterone (TT), free testosterone (FT) and estradiol (E2) were assessed. All subjects were administered ten standardized neuropsychological tests, with established reliability and validity, which tested cognition, verbal learning and memory, visual-motor and visual-spatial abilities, and graphomotor and psychomotor speed.

Our sample consisted of 160 men with a mean age of 43.3 years (SD 6.2), with 81% African Americans. We found that 28.1% had serum TT <300 ng/dl or FT <50 pg/ml. The mean level of TT was 539 ng/dL (SD 284.7), the median was 499 ng/dL (range 128-2050). The mean level of FT was 68 pg/mL (SD 34.5), the median was 66.5 pg/mL (range 10-157). The mean E2 was 2.8 pg/mL (SD 3.3), and the median was 2.1 pg/mL (range 0.5-26). The population studied was primarily uneducated, of low income, not married and unemployed. After adjustment for age, education, depression, and drug use category, we did not observe any statistically significant correlation between cognitive performance and sex hormone levels.

In this cross-sectional study of young and middle-aged men with a high prevalence of SUD and 28.1% with hypogonadism (serum T<300 ng/dl or FT <50 pg/ml), endogenous levels of TT, FT or E2 were not related to cognitive performance. Other factors need to be identified which contribute to poor administrative cognitive function in the setting of substance use.

Nothing to Disclose: MFZ, XX, AW, OS, ASD

Pub # P3-215

Session Information POSTER SESSION: CLINICAL - Male Reproductive Endocrinology II & Case Reports (1:30 PM-3:30 PM)

Title High Prevalence of Hypogonadism in Male Patients with Chronic Renal Failure

Author String W Reinhardt, V Bouronikou, S Dolf, M Broecker, K Mann, F Jockenhoewel, O Witzke
Dialysis Center, Herne, Germany; Ev Krankenhaus, Herne, Germany; University of Essen, Essen, Germany; University of Essen, Essen, Germany

Body

Introduction:
Chronic hemodialysis treatment is associated with a high prevalence of low testosterone levels in male patients. Moreover, clinical features of hypogonadism are similar to those with chronic renal failure (CRF). So far, there are only few data on the prevalence of hypogonadism in patients with mild or moderate CRF. Therefore, we prospectively investigated sex hormone status in a large patient cohort in various stages of CRF and in patients on chronic hemodialysis.

Methods:
280 male patients in CKD (chronic kidney disease) stage 1-5 and in 47 patients requiring chronic hemodialysis were prospectively investigated; besides clinical and renal data (such as BMI, waist circumference, serum creatinine, GFR, serum albumine) the following endocrine parameters in serum were measured: testosterone, free testosterone (calc. by the Vermeulen formula), bioavailable testosterone, SHBG and prolactin.

Results:
34% of all 280 patients in stage CKD 1-5 demonstrated biochemically hypogonadism, i.e. serum testosterone <10 nmol/l. 48 % of patients with CKD stage 4 and 5 (GFR < 30 ml/min) and 49 % of the dialysis patients had testosterone levels < 10 nmol/l. There was no difference in testosterone levels between those two groups; stage 4 and 5: 10.6 +/- 5.3 nmol/l (mean +/- SD) vs 10.5 +/- 5.1 in the dialysis group. There was a positive correlation of total and free testosterone and GFR ($r=0.25$ and $r=0.32$ respectively; $p<0.001$). Testosterone was negatively correlated with age ($r=-0.31$; $p<0.001$) and with waist circumference ($r=-0.24$; $p<0.001$). Prolactin was slightly but significantly higher in the dialysis group: 14.6 +/- 15.2 ng/ml vs 11.5 +/- 15.8 in CKD stage 4 and 5 ($p<0.015$).

Conclusion:
1/3 of male patients with CRF (stage 1-5) have testosterone deficiency. Decreasing renal function is associated with decreasing total and free testosterone levels. Even moderate to severe renal failure (CKD stage 4 and 5) is associated with a high prevalence of hypogonadism (up to 50 %) and is not different from a dialysis patient cohort.

Nothing to Disclose: WR, VB, SD, MB, KM, FJ, OW

Pub # P3-216

Session Information POSTER SESSION: CLINICAL - Male Reproductive Endocrinology II & Case Reports (1:30 PM-3:30 PM)

Title Testosterone Changes over Time in Men with Human Immunodeficiency Virus Infection: Preliminary Results

Author String G Brigante, D Santi, L Zirilli, C Diazi, G Orlando, VL Gnarini, C Carani, G Guaraldi, V Rochira
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Body **Introduction:** male hypogonadism is very frequent in men treated with highly active anti-retroviral therapy (HAART) for Human Immunodeficiency Virus-1 (HIV-1) infection, reaching a prevalence of about 20%. Literature data regarding the time course of serum total testosterone (T) levels in these patients are lacking. **Aim of the study:** to evaluate changes of T levels over time in HIV-positive men with an initial finding of low T (<300 ng/dl). **Methods:** measurement of T and serum LH in 111 hypogonadic HIV positive outpatients aged 31-68 years (mean 46.3 years) at baseline and after 12 months. **Results:** mean T value at baseline was 235.8 ng/dl. After 12 months, 36 subjects (32.4%) had no change in T (a variation of less than 50 ng/ml), 3 patients (2.7%) had a decrease (more than 50 ng/ml) and 72 patients (64.9%) had an increase in T (more than 50 ng/ml). 63 patients (56.7%) normalized T (>300 ng/ml). Mean T value at baseline of the 63 patients who restored T was 236.3±66.9 ng/ml, while mean T value at baseline of the 9 patients who didn't normalize was 151.4±86.5 ng/ml. **Conclusions:** most of the HIV patients under HAART with an initial finding of low T present normalized T in the following months. Baseline T values seem to be predictive of the future evolution of the disease, higher T level being associated with subsequent T normalization. In HIV patients a single finding of hypotestosteronemia needs further confirmation before starting androgen therapy and often a wait-and-see approach is mandatory.

Nothing to Disclose: GB, DS, LZ, CD, GO, VLG, CC, GG, VR

Pub # P3-217

Session Information POSTER SESSION: CLINICAL - Male Reproductive Endocrinology II & Case Reports (1:30 PM-3:30 PM)

Title Hypogonadism and Inflammation in Cancer Patients

Author String B Burney, J Garcia, T Hayes
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Body **OBJECTIVE:** To determine the prevalence of hypogonadism in male cancer patients and establish the relationship between testosterone and functional performance, sexual dysfunction, appendicular lean body mass (aLBM), muscle strength, fatigue and inflammatory markers in this population.
METHODS: Males with cancer-cachexia (CC, n=45), with cancer but without cachexia (CNC, n=45) and age, race, gender and BMI matched non-cancer controls (CO, n=45) were enrolled in this cross-sectional study. Total testosterone (TT), SHBG, albumin, CRP and IL-6 were measured in an early morning blood sample. Bioavailable testosterone (BT) was calculated using the mass-action equation. Functional performance was assessed by the ECOG (Eastern Cooperative Oncology Group) and Karnofsky performance scales (KPS). Fatigue was measured by FACIT-F (Functional Assessment of Chronic Illness Therapy-Fatigue) and sexual function was evaluated by IIEF (International Index of Erectile Function) and UCLA psychosexual scale. Body composition was measured by DEXA and muscle strength by handgrip strength (HS). Continuous variables were analyzed by ANOVA or Kruskal-Wallis tests adjusting for multiple comparisons (Tukey's test). Categorical data were compared using the X^2 test. Linear regression tested the predictive value of variables on erectile function, HS, aLBM, fatigue and performance. Individual predictors were analyzed separately and included in a multiple regression analysis if significant.
RESULTS: Cancer groups were matched for opioids use, cancer type, stage and chemotherapy use. TT was lower in CC compared to CNC ($p<0.05$) and BT was lower in CC in comparison to CO ($p<0.05$). Albumin was lower in CC compared to the other groups ($p<0.001$). IL6 and CRP were higher in CC than in CO ($p<0.05$). Also, CC had significantly lower grip strength ($p\leq 0.05$), erectile function as measured by IIEF, aLBM and fat mass ($p=0.05$) compared to CO. Performance status (ECOG and KPS) was lower in CC compared to CNC and CO groups ($p=0.05$). On multivariate analysis, grip strength and albumin predicted erectile function, TT and LBM predicted grip strength and grip and CRP predicted LBM. Albumin predicted performance.
CONCLUSION Patients with cancer cachexia have lower grip strength, testosterone, fat mass and aLBM. They also have higher CRP and IL6 and poor functional status and erectile function. There is a clear association between these laboratory markers and clinically meaningful outcomes in this population.

Sources of Research Support: Grant from Abbott pharmaceuticals.

Disclosures: JG: Researcher, Abbott Laboratories; Speaker, Bayer, Inc., Novo Nordisk. Nothing to Disclose: BB, TH

Pub # P3-218

Session Information POSTER SESSION: CLINICAL - Male Reproductive Endocrinology II & Case Reports (1:30 PM-3:30 PM)

Title Hypothalamic-Pituitary-Gonadal Axis Function in Men with Type 2 Diabetes Mellitus

Author String P Knoblovits, SM Suarez, HE Scaglia, C Zylbersztein, LE Litwak, PR Costanzo
Hospital Italiano, Buenos Aires, Argentina; CEUSA-LAEH, Buenos Aires, Argentina

Body Numerous studies shown that men with type 2 diabetes mellitus (DM2) have lower levels of testosterone (T) and a higher prevalence of hypogonadism. It still remains unclear the mechanism by which there is a relationship between hypogonadism and DM2. Obesity and insulin resistance are both postulated as causes of a decrease of testicular T synthesis. Aims: to evaluate the different levels of the hypothalamic pituitary gonadal axis in patients with DM2. Methods: Fourteen patients with DM2 at least for 6 months since diagnosis (DM2 Group) and 15 subjects without DM2 (normal glucose tolerance test) as control group (CG) were included. Laboratory: We assessed fasting glucose, insulin, HOMA (Homeostasis Model Assessment), total T (TT), bioavailable T (BT), free T (FT), estradiol (E2), bioavailable E2 (BE2) and sex hormone binding globulin (SHBG) levels, LH pulsatility through extractions of blood every 10 minutes for 4 hours, starting at 06: 00 a.m. GnRH test: basal LH, 30, 60 and 90 minutes after 100 ug of GnRH i.v. HCG test: TT, BT, FT, E2, BE2 and SHBG basal and 48 hours post 5000 IU HCG i.m. There were no differences in age, BMI (body mass index) and waist circumference between groups. Glucose was higher in the DM2 group vs. CG: 131.1 ± 25.5 vs. 99.1 ± 13.6 mg/dL, $p=0.0005$. There were no difference in basal insulin, HOMA, TT, BT, FT, E2, BE2, SHBG and LH levels between groups. The DM2 group had lower LH pulsatility vs. GC: 0.8 ± 0.8 vs. 1.5 ± 0.5 pulses, $p=0.009$. LH pulse amplitude differences was not found. A negative correlation was found between the number of pulses and glucose, $r:-0.39$, $p=0.03$ and also between the number of pulses and SHBG, $r:-0.39$, $p=0.05$. There were no differences in the values of LH 30, 60 and 90 minutes post GnRH test between groups. There were no differences in the levels of TT, BT, FT, E2, BE2 and SHBG post HCG test between groups. Conclusions: patients with DM2 showed lower hypothalamic pulsatility without changes at the pituitary response to stimulus with GnRH nor testicular response to stimulus with HCG. Glucose negatively correlated with the number of pulses which suggests a negative effect of hyperglycemia in the hypothalamic secretion of GnRH.

Nothing to Disclose: PK, SMS, HES, CZ, LEL, PRC

Pub # P3-219

Session Information POSTER SESSION: CLINICAL - Male Reproductive Endocrinology II & Case Reports (1:30 PM-3:30 PM)

Title Androgen Deficiency in Patients with Chronic Heart Failure and Relationship with Serum Androgen Levels and Indicators of Disease

Author String M Cetin, S Isik, ZG Cetin, M Sahin, U Ozuguz, H Cicekcioglu, O Ucar, D Berker, S Guler
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Body

Objective: The age-related decline of circulating anabolic sex hormones in men is associated with increased morbidity and mortality. We studied the prevalence of testosterone (T) deficiency and its relationship with clinical, laboratory and echocardiographical parameters in men with chronic heart failure (CHF).

Methods: Fifty patients with CHF having standard HF treatment with a functional capacity of 1-4 based on NYHA classification and 30 age-matched healthy men were included in the study. Patients with known hypopituitarism were not included in the study. The patients were examined by a physician at the baseline visit and information on medical history, medication, classification of CHF based on New York Heart Association (NYHA) functional class, heart rate and resting blood pressure were recorded. Serum IGF-I, brain natriuretic peptide (BNP), T, free T (FT), dehydroepiandrosterone sulfate (DHEAS), and sex hormone binding globulin (SHBG) levels were analyzed. The echocardiographic examination was carried out by a single cardiologist blinded to patient details.

Results: Mean age and BMI of patients and control group were similar [57.8±10.7 (38-81) vs 54.0±7.5 (43-70), (p=0.113); 26.0±3.4 (20.6-36.1) vs 25.7±2.7 (22.0-34.0), (p=0.644)]. Mean EF values of patients were lower (28.3±7.3% vs 65.5±4.7%, p<0.001) while mean BNP levels were higher than control group (2269.3±2628.5 vs 102.5±283.6, p<0.001). In the patient group while SHBG levels were higher (49.5±13.9 vs 39.0±5.7, p<0.001) T, FT and DHEAS levels were lower compared to control group (8.1±2.8 vs 11.7±3.7, p<0.001; 4.0±1.1 vs 5.0±0.5, p<0.001; 91.9±49.4 vs 150.6±59.1, p<0.001, respectively). Reduced FT was present in 48.0% of patients with CHF compared to 10.0% in control group (p<0.001). NYHA score and BNP levels correlated positively with hCRP and SHBG levels and negatively with T, FT ve DHEAS levels. In contrast EF value showed a negative correlation with hCRP and SHBG levels and a positive correlation with T, FT ve DHEAS levels. In regression analyses there was an independent relationship between EF values and hCRP, IGF-I ve FT levels. Furthermore, FT, T and DHEAS levels were lower in patients with severe CHF compared to patients with NYHA score I-II (4.3±1.5 vs 9.7±1.3, p<0.001; 2.4±0.7 vs 4.7±0.3, p<0.001; 38.9±21.9 vs 114.6±39.5, p<0.001).

Conclusion: Our data indicate that androgen deficiency in patients with CHF is associated with poor clinical course.

Nothing to Disclose: MC, SI, ZGC, MS, UO, HC, OU, DB, SG

Pub # P3-220

Session Information POSTER SESSION: CLINICAL - Male Reproductive Endocrinology II & Case Reports (1:30 PM-3:30 PM)

Title Erectile Dysfunction Does Not Mirror Endothelial Dysfunction in HIV-Infected Patients

Author String D Santi, M Beggi, G Brigante, S Zona, K Luzy, G Orlando, R Rossi, P Bouloux, G Guaraldi, V Rochira
Department of Medicine, Endocrinology & Metabolism, Geriatrics, Modena, Italy; Department of Medicine & Medical Specialties, Modena, Italy; Department of Emergency, University of Modena and Reggio Emilia, Modena, Italy; Department of Medicine, Emory University, Atlanta, GA

Body
Background: the penis has been compared to a barometer of endothelial health, being erectile dysfunction (ED) an early sign of endothelial dysfunction. The aim of the study was to investigate the extent of the association between ED and endothelial dysfunction in patients with HIV infection on ART.
Methods: in this observational cross-sectional study we evaluated the prevalence and factors associated with ED in a cohort of 133 HIV-infected men. Evaluation tools included: the International Index of Erectile Function, ultrasound assessment of brachial artery flow mediated dilatation (FMD) and multi-slice computed tomography for coronary artery calcifications (CAC) as surrogates of endothelial dysfunction, the ATP III criteria to diagnose metabolic syndrome, plasma total testosterone (hypogonadism), and a visual analogue scale (VAS) of aesthetic satisfaction of the face and of the body (psychological distress associated with lipodystrophy).
Results: Thirty-nine (29.32%) patients had mild, 14 (10.52%) moderate and 26 (19.55%) severe ED. Prevalence of ED ranged from 45% to 65% respectively in patients less than 40 and more than 60 years old. Metabolic syndrome (MS) was present in 20 (25%) patients with ED and 13 (24%) without (p-value=0.87). Prevalence of ED did not appear to be associated with MS as a single clinical pathological entity, nor with the numbers of its diagnostic components. FMD < 7% was present in 25 (32%) patients with and 18 (33%) without ED (p-value=0.83) and CAC >100 was present in 8 (10%) patients with and 5(9%) without ED (p-value=0.87). A stepwise multivariable logistic regression analysis was used to find predictors of ED. Independent predictor were VAS face (OR=0.85, 95% CI 0.73-0.99, p=0.049) and age, per 10 years of increase (OR=1.73, 95% CI 1.02-2.94, p=0.04).
Conclusions: age constituted the most important risk factor for ED, which was related with aesthetic dissatisfaction of the face leading to negative body image perception.

Nothing to Disclose: DS, MB, GB, SZ, KL, GO, RR, PB, GG, VR

Pub # P3-221

Session Information POSTER SESSION: CLINICAL - Male Reproductive Endocrinology II & Case Reports (1:30 PM-3:30 PM)

Title Profile of Erectile Dysfunction (ED) at a Midwestern Endocrine Clinic

Author String SK Mishr, TW Church, KS Casukhela, A Pandey
ST Ritas Medical Center, Lima, OH; St Ritas Medical Center, Lima, OH; Ohio State University at Lima, Lima, OH; St Ritas Medical Center, Lima, OH

Body Background: There is need to identify treatable causes of ED. New treatments are needed for ED cases with generalized atherosclerosis of pudendal artery.
Evaluation of erectile dysfunction (ED); Chart analysis of 31 cases of erectile dysfunction between 6-3-2009 and 1-3-2011 referred to Endocrinology Clinic were evaluated for causes of ED. 97% had abnormal penile Doppler test. Average age 55.16 years (33 to 75 years). 60% cases had hypertension. 43% cases had hypothyroidism. 80% cases had abnormal lipids. 30% cases had hypogonadism. CT Angiogram of pelvis was abnormal in 36.67% cases. 40% had LDL levels above 100 mg% (<100 mg%). Coronary artery disease in 29% cases.
Androgen Deficiency in the Aging Male (ADAM) questionnaire is not a reliable tool for predicting testosterone levels. Out of cases positive on ADAM questionnaire 47% had total testosterone above 300 ng/dl (300 to 890 ng/dl). Positive penile Doppler had a good predictive value for pudendal artery atherosclerosis. We believe that testosterone levels are negatively associated with high values of BMI index, TSH and LDL, and positively associated with high HbA1c levels. A regression model was fit to examine the degree to which these variables are correlated with Testosterone levels.
Adjusted R-square is 32%, meaning that the explanatory variables account for 32% of the variation in Testosterone levels. While LDL and HbA1c are not significant, both BMI and TSH, with negative coefficients, are significant determinants of testosterone levels.
Conclusion: Total testosterone should be done in each case of ED. Hypogonadism, hypertension, coronary artery disease and Atherosclerosis is associated in most cases of ED.

Nothing to Disclose: SKM, TWC, KSC, AP

Pub # P3-222

Session Information POSTER SESSION: CLINICAL - Male Reproductive Endocrinology II & Case Reports (1:30 PM-3:30 PM)

Title Markers of Endocrine and Exocrine Gonadal Status in Men with Anorexia Nervosa

Author String B Estour, N Germain, Y Khalfallah, B Galusca
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Body

Background. The impact of undernutrition on endocrine and exocrine gonadotrope function is poorly known in male anorexia nervosa (AN) patients. The aim of the current study was to compare the pituitary gonadal function of male AN subjects with that of healthy controls, Kallmann syndrome (KS) patients and female AN subjects.

Subjects and Methods. Hormonal parameters including FSH, LH, SHBG, oestradiol, testosterone, inhibin B, thyroid hormones, GH, IGF-I, cortisol, ACTH, DHEAS and leptin were assessed in 30 male and 22 female subjects with restrictive-type AN, 22 male and 20 female controls, and 9 male KS patients.

Results. Similar abnormalities of free T3, GH, IGF-I, cortisol and leptin were found in men as in AN women with equivalent undernutrition status when compared with corresponding controls. Low levels of LH, FSH were found in both male and female AN patients. In male AN, total testosterone and free testosterone index were found lower than in controls but higher than in KS, while a lack of oestradiol was noticed in AN women. Sex hormones variations were directly related to weight gain only in AN men. No relationship was found between sex hormones and leptin variation for both sexes. In AN men, inhibin B levels were similar to that of controls and did not correlate with testosterone levels.

Conclusions. Significant differences of undernutrition impact on gonadal status were noticed between male and female AN subjects, including partial preservation of testosterone release and probable preservation of exocrine function, according to the normal inhibin B levels.

Nothing to Disclose: BE, NG, YK, BG

Pub # P3-223

Session Information POSTER SESSION: CLINICAL - Male Reproductive Endocrinology II & Case Reports (1:30 PM-3:30 PM)

Title Changes in Semen Quality during Young Adulthood -- Longitudinal Follow-up of Two Finnish Cohorts

Author String A Perheentupa, R Ronka, HE Virtanen, M Vierula, J Matomaki, N Jorgensen, NE Skakkebaek, J Toppari
University of Turku, Turku, Finland; Rigshospitalet, Copenhagen, Denmark

Body

Objective: To study whether semen quality changes in early adulthood (19 - 29 years).

Design: Longitudinal follow up of two cohorts of young adult Finnish men.

Setting: University Andrology Research Unit.

Subjects: Two cohorts of voluntary men followed up from the age of 19 years onward.

Intervention: Semen analysis of the participating men was performed at two to five year intervals over a period of five (cohort B) to ten (cohort A) years. Physical examination was carried out at each visit to rule out any significant andrological abnormalities.

Main Outcome Measure: semen variables: volume, sperm concentration, total sperm count, motility and morphology.

Results: 336 men in the cohort A participated in the first round of the study, and in the final fourth round 111 men were examined. 197 men attended the first visit in the cohort B, and in the final third visit 96 men were evaluated. Neither sperm concentration (median 60 and 50 million/mL in A and B, respectively at the age of 19; 70 and 46 at the end) nor the total sperm count (193 and 172 million in A and B at 19; 219 and 167 at the end, respectively) changed significantly during the follow up. However, the percentage of motile sperm increased (66 and 75 % motile sperm in A and B at 19; 82 and 82 % at the end, respectively). Percentage of sperm with normal morphology also increased during the follow up, as judged on the 61 men that participated in all 4 follow-up visits of the cohort A: 7.5 - 7.0 - 8.0 - 10.0 % at 19, 21, 24, and 29 years of age.

Conclusion(s): Full spermatogenic capacity is reached before the age of 19 years and thereafter sperm production capacity does not significantly improve. However, both sperm motility and percentage of structurally normal sperm increase during young adulthood. Thus, semen quality of 19-year-old men reflects well quantitative capacity of spermatogenesis, but sperm quality may improve during further maturation.

Sources of Research Support: Academy of Finland, Sigrid Juselius Foundation, Turku University Hospital and EU Fp7 Environment DEER.

Nothing to Disclose: AP, RR, HEV, MV, JM, NJ, NES, JT

Pub # P3-224

Session Information POSTER SESSION: CLINICAL - Male Reproductive Endocrinology II & Case Reports (1:30 PM-3:30 PM)

Title High Prevalence of Micropenis and Cryptorchidism in Brazilian Patients with Congenital Hypogonadotropic Hypogonadism: Impact of Testosterone Replacement Therapy Started in Adolescence or Adulthood on Final Penile Length

Author String AM Faria, LFG Silveira, MG Teles, AP Abreu, LP Brito, S Domenice, AC Latronico, EMF Costa, BB Mendonca
Hospital das Clinicas, Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil

Body
Background: Micropenis and cryptorchidism have been classically associated with congenital isolated hypogonadotropic hypogonadism (IHH). A previous study reported low prevalence of micropenis and cryptorchidism in IHH, lacking sensitivity to aid in the clinical diagnosis of this disorder (1).
Aim: To evaluate the prevalence of micropenis and cryptorchidism in a Brazilian cohort of IHH without any prior hormonal therapy and the impact of testosterone replacement started in adolescence or adulthood on penile growth, as well as the presence of pretreatment gynecomastia.
Patients and methods: Penile length was measured as previously described (2) and compared to Brazilian standards (3). Micropenis was defined as a morphologically normal penis, with a stretched length of more than -2.5 SD below the mean value for age. Nineteen patients with Kallmann's syndrome (KS) and 10 patients with normosmic IHH (nIHH) had basal pretreatment and follow-up measures of penile length after at least 1 year of full dose intramuscular testosterone esters replacement. Mean age of initiation of therapy was 20.5 years in KS (13-41) and 20 years in nIHH (16-26). Basal median LH and total testosterone was <0.6 IU/l (<0.6-0.9) and 33.5 ng/dl (11-84) in KS, and <0.6 IU/l (<0.6-1.3) and 27 ng/dl (<14-43) in nIHH, respectively
Results: A history of cryptorchidism was present in 63% of patients with KS and 30% with nIHH and pretreatment gynecomastia in 26% with KS and in 20% with nIHH. All patients had basal penile length compatible with micropenis. Basal average penile length was 5.63 cm (-5.17 SD) in patients with KS and 5.71 cm (-5.44 SD) in patients with nIHH. Post-treatment average penile length was 9.81 cm (-2.93 SD) in KS (gain of 4.17 cm or +2.24 SD, prevalence of micropenis=68%) and 9.87 cm (-2.89 SD) in nIHH (gain of 4.16 cm or +2.55 SD, prevalence of micropenis=50%).
Conclusions: Micropenis and cryptorchidism are highly prevalent conditions in patients with IHH. This is in accordance with the knowledge that fetal pituitary LH is important for penile growth from midgestation to birth and during the postnatal surge of testosterone during the first 4-6 months of life (2). Testosterone replacement in adolescence or adulthood markedly improves penile length, although micropenis persists in the majority of patients, mainly in KS. These findings suggest that micropenis and cryptorchidism are important clinical parameters for distinction between IHH and constitutional delay of puberty.

(1) Pitteloud N et al. The role of prior pubertal development, biochemical markers of testicular maturation, and genetics in elucidating the phenotypic heterogeneity of idiopathic hypogonadotropic hypogonadism. *J Clin Endocrinol Metab.* 2002; 87:152-60.
(2) Tsang S. When size matters: a clinical review of pathological micropenis. *J Pediatr Health Care.* 2010; 24:231-40.
(3) Gabrich PN et al. Penile anthropometry in Brazilian children and adolescents. *J Pediatr (Rio J).* 2007; 83:441-6.

Nothing to Disclose: AMF, LFGS, MGT, APA, LPB, SD, ACL, EMFC, BBM

Pub # P3-225
Session Information POSTER SESSION: CLINICAL - Male Reproductive Endocrinology II & Case Reports (1:30 PM-3:30 PM)
Title A Patient with Kallman Syndrome and a Rare Craniofacial Defect
Author String B Alfonso, R Bier, T Araki, A Busta
Beth Israel Medical Center, New York, NY; Beth Israel Medical Center, New York, NY

Body

Introduction:

We present a rare case of Kallmann Syndrome (KS) and platybasia.

Case presentation:

A 26-year-old Chinese man with a history of cleft palate, presented with high pitched voice, low energy, decrease libido, and no erections. Family history was noncontributory. Physical exam revealed eunuchoidal appearance, arm span greater than the height, small left testicle, right cryptorchidia, micropenis and no axillary or pubic hair. Laboratory tests confirmed idiopathic hypogonadotropic hypogonadism (IHH): LH <0.1 (n 5-9.3 mIU/mL), FSH <0.7 (n 6-8.0 mIU/mL), total testosterone 19 ng/dl (n 250-1100 ng/dL), free testosterone 1.1pg/ml (n 35-155pg/mL). Other pituitary function tests were normal. Ultrasound was unable to detect the right testis. MRI of the brain revealed platybasia of skull base and no evidence of pituitary adenoma. Upon further questioning he admitted to having anosmia, which was confirmed by ENT testing. Kallman syndrome (KS) was diagnosed. After starting testosterone replacement, patient noticed improved erections, energy, muscle strength and increased body hair.

Discussion:

IHH is a condition characterized by low gonadotropins and testosterone, no hypothalamic or pituitary abnormalities, and is often a clinical diagnosis at the age of 18 with the absence or lack of complete sexual maturation (1). The diagnosis is often delayed until puberty (3). The association of gonadotropin-releasing hormone (GnRH) deficiency with anosmia or hyposmia is known as KS. The incidence of KS is 1 in 10,000 men and 1 in 50,000 women (2). Mutations in at least six genes have been identified for KS, with a reported sensitivity of 30% (3). IHH is often associated with a variety of anomalies such as anosmia/hyposmia, cleft palate, dental agenesis, visual abnormalities, deafness, mental retardation, renal agenesis, pes cavus, cerebella dysfunction, and synkinesia. (4) Our patient had Kallmann syndrome associated with platybasia, which has not been described in the literature.

Conclusion:

IHH is a rare disorder typically associated with a variety of anomalies. A presentation of delayed puberty, in a patient with eunuchoidal appearance and high-pitched voice should prompt a work-up of Kallman syndrome. A rare finding in these patients is platybasia, and other craniofacial defects may be prevalent among patients with Kallmann syndrome.

(1) Raivio et al., N Engl J Med 2007; 357:863-873

(2) Qu et al., J Otolaryngol Head Neck Surg, 2010; 39 (6):723-731(3) Kaplan et al., Am J Med Genet A. 2010; 152A(11):2796-2801

(4) Layman et al., Endocrinol Metab Clin N Am 2007; 36:283-296

Nothing to Disclose: BA, RB, TA, AB

Pub # P3-226

Session Information POSTER SESSION: CLINICAL - Male Reproductive Endocrinology II & Case Reports (1:30 PM-3:30 PM)

Title Hormonal Response to Weight Gain in a Male with Low Fat Mass, Relative Under-Nutrition and Over-Training

Author String NM Neary, AB Courville, S Smith, GR Hall, KY Chen, SC Monica
 Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD; National Institutes of Health, Bethesda, MD; National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD

Body **Introduction:** Males who seek to increase muscle mass through exercise and caloric restriction may have low fat mass despite normal BMI.
Case: A 26-year old male was evaluated for fatigue, hypogonadism and GH deficiency. From age 19 he had followed a strict diet and exercise regime including low carbohydrate and high protein intake, and weight lifting for 1 hour 5 days a week with the aim of increasing muscle mass. Two years prior, he was evaluated and treated for testosterone and GH deficiency. To investigate the etiology of these abnormalities, we discontinued exogenous testosterone and GH. Three months later, he felt more fatigued with reduced libido. On examination he was muscular; height was 175cm, weight 63kg and BMI 20.5kg/m². The testicles measured 15cm³ bilaterally and felt soft. Biochemistry off testosterone therapy confirmed hypogonadotropic hypogonadism (FSH 11.6 (1-11U/l), LH 1.5 (1-8U/l), free testosterone 6.7 (9-30ng/dl)) but the growth axis was normal. Thyroid function tests were consistent with sick-euthyroidism (TSH 1.23, free T₄ 0.8 (0.8-1.5ng/dl), T₃ 46 (90-215ng/dl)). 24-h urinary free cortisol (UFC) were markedly elevated at 142 and 198 [mu]g/24h (3-45). SHBG and CBG were normal. Whole body DEXA revealed low fat mass (4.1kg, 6% body weight), and serum leptin was low at 1.6 (1.8-5.6[mu]g/l). Urinary synthetic androgen screening was negative. Food records and 24-hour energy expenditure showed mild caloric restriction. We hypothesized that the cause of hormonal derangement was relative hypoleptinemia and low fat mass secondary to caloric restriction and vigorous anaerobic exercise.
Intervention: We aimed to increase fat mass by 50% through greater energy intake, and gave him specific dietary recommendations. He continued the same training schedule.
Outcome: The patient gained 5.4kg in the next 4 months and felt better with more energy. His libido returned to normal. The testicles increased to 25cm³ bilaterally and felt buoyant. Fat mass increased to 7.5kg (11% body weight). Testosterone was normal (free testosterone 12.3ng/dl, FSH 9.3U/l, LH 2.9U/l). T₃ had increased to 67ng/dl but had not normalized. Overall UFCs had reduced (95 and 146[mu]g/24h), but remained elevated. The patient continues to struggle with body image issues.
Conclusion: Males with normal BMI who seek to increase muscle mass by caloric restriction and vigorous exercise are at risk of significant morbidity and hormonal derangement, which may be reversible by increasing body fat.

Nothing to Disclose: NMN, ABC, SS, GRH, KYC, SCM

Pub # P3-227
Session Information POSTER SESSION: CLINICAL - Male Reproductive Endocrinology II & Case Reports (1:30 PM-3:30 PM)
Title Ambiguous Genitalia in a Newborn with Spironolactone Exposure
Author String A Shah
University of Texas at Houston, Houston, TX

Body

Background:

The anti-androgen effects of spironolactone are known, however exposure has not been reported to be associated with ambiguous genitalia.

Clinical Case:

A term, 2 day old infant presented with ambiguous genitalia. The mother was on spironolactone for treatment of PCOS until 5 weeks gestation. The perinatal course was significant for ambiguous genitalia diagnosed on prenatal ultrasound and XY genotype by amniocentesis. Examination showed a 2.2 cm webbed penis with suprapubic fat pad and tight phimosis with functioning external urethral meatus. The scrotum was well pigmented, testes were bilaterally high riding.

Laboratory testing performed at 2 days showed an elevated TSH (23.6 uIU/ml), consistent with postnatal surge, elevated testosterone (542 ng/dl) corresponding with physiological GnRH surge, and normal ACTH (37 pg/ml) and cortisol (9.5 ug/dl). 17 OH progesterone was ordered but not completed and 17 OH pregnenolone was normal (546 ng/dl). Postnatal chromosomes were also 46, XY. Repeat labs at 1 month showed normalization of TSH (2.3 mIU/L) and testosterone (234 ng/dl). 17OH progesterone (39 ng/dl) and 17OH pregnenolone (185 ng/dl) at 2 months were also normal ruling out 3-beta hydroxysteroid deficiency. DHT (57 ng/dl) and testosterone (202 ng/dl) were also performed and revealed a T:DHT ratio of 3.5, ruling out 5-alpha reductase deficiency. We also recommended genetic evaluation for partial androgen insensitivity if another etiology was not found, which is pending.

Discussion:

Spironolactone has been used for its anti-androgen effects for treatment of PCOS. Also it has been shown to cause gynecomastia in males. A literature review reveals previous studies of spironolactone effects on heart disease in infants, but no reports regarding sexual differentiation disorders of infants with spironolactone exposure. An animal study from 1980 showed that pregnant rats treated with spironolactone from day 13-21 post conception had male fetuses with signs of feminization of the external genitalia (1). However a previous study in 1975 in which pregnant rats were given spironolactone from day 14 to delivery did not show any evidence of feminization (2).

Conclusion:

We should use caution in treating females of child-bearing age with spironolactone as the anti-androgen effects of this medication could have impact on sexual differentiation of the infant.

(1) Hecker A, Hasan SH, Neumann F. Disturbances in sexual differentiation of rat foetuses following spironolactone treatment. *Acta Endocrinologica (Copenhagen)*. 1980 Dec;95(4):540-5.

(2) Rose LI, Regestein Q, Reckler JM. Lack of effect of spironolactone on male genital development. *Investigative Urology* 1975;13(2):95-6.

Nothing to Disclose: AS

Pub # P3-228

Session Information POSTER SESSION: CLINICAL - Male Reproductive Endocrinology II & Case Reports (1:30 PM-3:30 PM)

Title Iatrogenic Polycythemia Vera Caused by Clomiphine Citrate Therapy of Hypogonadal Men

Author String N Onwubalili, MM-H Cho
UMDNJ-New Jersey Medical School, Newark, NJ; University Reproductive Associates, PC, Hasbrouck Heights, NJ

Body

Case 1. M.D. is a 33 year old man referred with his wife for evaluation of infertility. M.D. was found to have oligo-terazoospermia. Urology noted that the examination was unremarkable. M.D. was initiated on clomiphine citrate (CC) 25mg daily. At 3 months follow-up, MA's hematocrit increased from 48.0% to 54.1% as total testosterone increased from 216 ng/dL to 507 ng/dL.
Baseline: FSH 5.4 mIU/ml, LH 4.8 mIU/ml, estradiol 36 pg/ml, total testosterone 216 ng/dL.
After therapy: FSH 7.3 mIU/ml, LH 11.4 mIU/ml, estradiol 19.1 pg/ml, testosterone 581 ng/dL.

Case 2. D.L. is a 42 year old man referred with his wife for evaluation of secondary infertility. D.L. reported a history of "low testosterone" and has been on intramuscular testosterone therapy. D.L. was found to have severe oligo-astheno-terazoospermia. His hematocrit was 50.1%. D.L.'s testosterone therapy was discontinued, and his hematocrit decreased to 46.1%, and testosterone was 163 ng/dL. D.L. was then started on CC 25mg daily and his testosterone increased to 487 ng/dL and 47% after the first month of therapy. In the third month of CC therapy, D.L.'s testosterone was 716ng/dL and hematocrit increased to 53.5%.

Background:
A known side-effect of testosterone therapy is polycythemia vera (PV). The Endocrine Society recommends that patients on testosterone therapy have a baseline hematocrit, another one at 3-6 months, with subsequent annual testing thereafter while on testosterone therapy. Discontinuation of testosterone therapy is recommended in patients with a hematocrit >54%.
CC is a selective estrogen receptor modulator that increases production of gonadotropins by inhibiting negative feed back to the hypothalamus and pituitary. CC is used off-label for treatment of male hypogonadism (low testosterone and/or oligozoospermia). Men treated with CC have variable response to the medication. Those that respond to CC therapy have elevated levels of FSH and/or LH. Higher level of LH often results in higher levels of testosterone, some with supra-physiologic levels of testosterone. However, men who receive CC therapy are not routinely evaluated for PV. Our cases demonstrate that PV can result from "normal" levels of testosterone in a previously hypogonadal male treated with CC.

Conclusion:
CC causes an increase in hematocrit in men, similar to testosterone therapy. Men treated with CC should have hematocrit monitored regularly for the development of PV.

Nothing to Disclose: NO, MM-HC

Pub # P3-229

Session Information POSTER SESSION: CLINICAL - Male Reproductive Endocrinology II & Case Reports (1:30 PM-3:30 PM)

Title Pulsatile Subcutaneous GnRH Therapy Produced a Pregnancy without [italicIn Vitro Fertilization for a Patient with Hypothalamic Hypogonadism Due to Craniopharyngioma

Author String M Iwabuchi, H Miyake
Seirei Mikatahara General Hospital, Hamamatsu, Japan; Hamamatsu Rosai Hospital, Hamamatsu, Japan

Body OBJECT: Craniopharyngiomas frequently grow on the cisternal surface of the hypothalamic region. Patients with craniopharyngioma may present at any age with partial or complete pituitary insufficiency. The ensembles of gonadotropin-releasing hormone (GnRH) neurons in hypothalamus that send axons to portal blood system in median eminence fire in coordinated, repetitive, episodic manner, producing distinct pulses of GnRH in the portal blood system. We experienced the case of hypothalamic hypogonadism after the radical surgery of craniopharyngioma located in the right basal ganglia and hypothalamus. During the operation, pituitary gland and stalk were preserved. The patient had developed panhypopituitarism and hypothalamic hypogonadism after transcranial surgery. The diagnosis of oligospermia and asthenospermia was made. We hypothesized that the hypogonadism was due to the lack of distinct pulses of GnRH after the surgery. The aim of this program was to demonstrate the efficacy of the pulsatile subcutaneous LH-RH therapy for the recovery of gonadal function from hypothalamic hypogonadism.
METHOD: Low dose LH-RH treatment was given to a 29-year-old male with small portable automatically-timed infusion pump, connected to a subcutaneous (s.c.) catheter. Gonadorelin acetate was prepared to the concentration of 0.8mg/ml. Sixteen micrograms of LH-RH s.c. was given every 120 min. Serum levels of gonadotropin and testosterone were monitored. The treatment was continued until his gonadal function normalized. This program was performed with informed consent. Long-term follow up has continued after low dose LH-RH treatment.
RESULT: After the six months of pulsatile LH-RH treatment, serum level of testosterone turned normal. The treatment was paused in 2003. His wife became pregnant with in-vitro fertilization (IVF) in 2007 and gave birth in 2008. Moreover, his wife became pregnant naturally without IVF in 2009 and gave birth in 2010, suggesting the recovery from oligospermia and asthenospermia of her husband.
CONCLUSION: We demonstrated a case of successful treatment of the hypothalamic hypogonadism after the surgery of craniopharyngioma. The pulsatile subcutaneous LH-RH therapy is efficacious for the case with the lack of the distinct pulses of GnRH after radical surgery of craniopharyngioma. The pulsatile subcutaneous GnRH therapy produced a pregnancy without in-vitro fertilization for a patient with hypothalamic hypogonadism due to craniopharyngioma.

Nothing to Disclose: MI, HM

Pub # P3-230

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Influences on Programming of Neuroendocrine Pathways (1:30 PM-3:30 PM)

Title Prenatal Androgen Exposure Alters Hypothalamic Metabolic Control Neurons in an Animal Model of PCOS

Author String RC Phillips, V Padmanabhan, L Coolen, M Lehman
University of Western Ontario, London, Canada; University of Michigan, Ann Arbor, MI; University of Michigan, Ann Arbor, MI; University of Michigan, Ann Arbor, MI; University of Michigan, Ann Arbor, MI; University of Michigan, Ann Arbor, MI

Body Polycystic ovarian syndrome (PCOS) is a major cause of infertility in reproductive-aged females and is closely associated with obesity and metabolic disease. Over the last few years, accumulating evidence suggests that prenatal exposure to excess steroid hormones can markedly increase the risk of PCOS, and its reproductive and metabolic deficits. Using the prenatal testosterone (T)-treated female sheep as a model for PCOS, we have found permanent changes in reproductive neuroendocrine neurons that may underlie the functional reproductive deficits seen in these animals (1). In addition, we recently found that the neuronal circuitry involved in mediating appetite regulation and energy homeostasis in the hypothalamus is also altered by prenatal exposure to T. Specifically, prenatal T-treated animals had nearly double the number of appetite stimulatory AgRP-expressing neurons in the middle division of the arcuate nucleus (ARC) of the hypothalamus compared to control animals, but no change in the number of appetite suppressing POMC-expressing neurons. Thus, excess androgen exposure during fetal life in the female sheep permanently alters the number of AgRP neurons and their projections, suggesting a possible mechanism by which prenatal T may contribute to insulin resistance and an increased risk of obesity in PCOS women. Because prenatal T sheep are hyperinsulinemic, and insulin normally acts upon hypothalamic neurons to decrease AgRP expression, we hypothesized that the increased AgRP expression seen in prenatal T sheep is due to decreased expression of insulin receptors within AgRP neurons. Dual-label immunocytochemistry and confocal microscopy were used to examine insulin receptor expression in AgRP and POMC neurons between prenatal T (n=5) and control ewes (n=5). Indeed, in the middle ARC prenatal T-treated animals had a significantly decreased percentage of AgRP neurons that express insulin receptors (71 ± 3.6) compared with controls (84 ± 1.7). This was also observed in the caudal ARC (prenatal T: 68 ± 6.6 ; Control: 81 ± 6.3). In contrast, no differences in the percentage of POMC neurons that express insulin receptors were detected. Overall, these results may lay the foundation for manipulation studies that will attempt to reverse the metabolic peptide imbalance and restore normal energy balance to prenatal T-treated animals and help address the metabolic deficits seen in women with PCOS.

(1) Cheng et al., *Endocrinology* 2010; 151

Sources of Research Support: NIH R01 HD041098 to V.P. and P01 HD044232 to V.P., M.N.L., and L.M.C.

Nothing to Disclose: RCP, VP, LC, ML

Pub # P3-231

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Influences on Programming of Neuroendocrine Pathways (1:30 PM-3:30 PM)

Title Analysis of Specific Brain Regions Responsible for the Parent-of-Origin Effects of $G_{s\alpha}$ Mutation on Metabolic Regulation

Author String A Berger, M Chen, J Wang, LS Weinstein
NIDDK, NIH, Bethesda, MD

Body Central melanocortins promote negative energy balance (reduced food intake, increased energy expenditure) and melanocortin receptor MC4R mutations lead to obesity and glucose intolerance/insulin resistance in humans and mice. Albright hereditary osteodystrophy (AHO) is a monogenic obesity disorder caused by heterozygous mutations of the ubiquitously expressed G protein $G_{s\alpha}$ that couples receptors including MC4R to intracellular cAMP generation. In AHO obesity develops only when the mutation is on the maternal allele. Likewise, mice with maternal (but not paternal) heterozygous germline $G_{s\alpha}$ mutations develop obesity and diabetes primarily due to lower sympathetic nerve activity (SNA) and energy expenditure with no change in food intake. We have shown in heterozygous brain-specific $G_{s\alpha}$ knockout mice (BrGsKO) that these parent-of-origin effects are due to $G_{s\alpha}$ imprinting within the central nervous system (CNS) and that $G_{s\alpha}$ is imprinted in the paraventricular nucleus of the hypothalamus (PVN) being preferential expressed in the maternal allele (1). Mice with maternal $G_{s\alpha}$ mutation restricted to PVN (mPVNGsKO) generated using Sim1-cre mice also developed obesity and insulin resistance, but the effect was much milder and was more obvious in males. In addition, whereas maternal BrGsKO mice had impaired stimulation of energy expenditure in response to an MC4R agonist and reduced catecholamine levels and *Ucp1* expression in brown adipose tissue indicative of reduced SNA, we did not observe similar changes in mPVNGsKO mice. Therefore $G_{s\alpha}$ imprinting in PVN does not fully account for the metabolic phenotype caused by maternal $G_{s\alpha}$ mutations and $G_{s\alpha}$ is likely to be also imprinted in other CNS regions involved in metabolic regulation where MC4R is expressed. Mice with $G_{s\alpha}$ mutation limited to the ventral medial hypothalamus (VMH) generated using Sf1-cre mice showed no metabolic phenotype and no molecular evidence for $G_{s\alpha}$ imprinting in VMH. The rostral raphe pallidus (rRPa) is a brainstem region that is proposed to contain sympathetic premotor neurons for innervation to brown adipose tissue and is required for induction of thermogenesis by melanocortins in the CNS (2). We now show that $G_{s\alpha}$ is imprinted in rRPa with maternal $G_{s\alpha}$ mutations leading to significantly lower $G_{s\alpha}$ expression in rRPa as compared to paternal $G_{s\alpha}$ mutations. Therefore $G_{s\alpha}$ deficiency in rRPa may also be an important contributor to the parent-of-origin effect of $G_{s\alpha}$ mutations on energy homeostasis.

1. Chen M et al., Cell Metab 2009; 9:548.
2. Fan W et al., Brain Res 2007; 1179:61

Sources of Research Support: Intramural Research Program, NIDDK, NIH.

Nothing to Disclose: AB, MC, JW, LSW

Pub # P3-232

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Influences on Programming of Neuroendocrine Pathways (1:30 PM-3:30 PM)

Title Effects of Maternal Starvation on the Offspring's Metabolism

Author String M Vujovic, B Vennstrom, J Mittag
Karolinska Institutet, Stockholm, Sweden

Body During pregnancy, maternal factors such as nutrients or hormones control the development of the embryo. Even minor disturbances in these fetal programming effects have consequences for the offspring, which often persist into adulthood. Disrupted fetal programming is also responsible for the hypermetabolic phenotype of mice with a mutant thyroid hormone receptor alpha 1 gene (TRa1+m mice). These mice display hyperactive sympathetic nervous system, which stimulates the brown adipose tissue to waste energy. To define the consequence of maternal hypermetabolism during pregnancy we analyzed the metabolic phenotype of wildtype offspring born by TRa1+m mothers (ms wt). Our results showed that ms wt male mice displayed reduced body weight after 7 weeks of age when compared to wt controls born by the wt mothers. This reduction was accompanied by 65% reduction in total fat mass as indicated by a micro-MRI scan analysis. Using hepatic glycogen as metabolic readout, we showed that ms wt animals had 50% lower glycogen content in the liver when compared to the controls, in addition to increased gluconeogenesis over glycolysis, as identified by hepatic mRNA levels. Furthermore, an intraperitoneal glucose tolerance test showed that ms wt animals exhibited an accelerated glucose clearance when compared to controls. Taken together, our results demonstrate a higher metabolic rate in these animals, indicating that maternal metabolism epigenetically programs offspring energy metabolism.

Nothing to Disclose: MV, BV, JM

Pub # P3-233

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Influences on Programming of Neuroendocrine Pathways (1:30 PM-3:30 PM)

Title Pubertal Lipopolysaccharide Treatment Blocks the Anti-Depressive Action of Estradiol in Adult Female Mice

Author String N Ismail, KM Olesen, PH Garas, R Rogan, S Servettalab, JD Blaustein
University of Massachusetts, Amherst, MA

Body Puberty is a period characterized by brain reorganization that contributes to the development of neural and behavioral responses to gonadal steroids. Previously, our laboratory has shown that a single injection of the bacterial endotoxin, lipopolysaccharide (LPS), during puberty decreased sexual receptivity in response to estradiol and progesterone. These findings suggest that pubertal LPS treatment alters the responsiveness to gonadal steroid hormones in adulthood. Since chronic estradiol treatment alleviates depression-like symptoms in ovariectomized adult mice, we were interested in investigating the effect of pubertal LPS treatment on estradiol's anti-depressive effects. We hypothesized that estradiol would not be effective at reducing depression-like behavior in ovariectomized female mice treated pubertally with LPS. Six-week (pubertal experimental group) and ten-week (adult control group) old female mice were injected with either saline or LPS. Five weeks later, they were ovariectomized and subcutaneously implanted with either an oil- or estradiol-filled Silastic capsule. Following one week of recovery, behavioral testing for depression-like symptoms began. The duration of immobility during the tail suspension and forced swim tests was used as a measure of behavioral despair, an important symptom of the depressive state. As expected, estradiol treatment decreased depression-like behavior in saline-treated mice and in mice treated with LPS at ten weeks of age. However, in mice treated with LPS at six weeks of age, estradiol strikingly increased the duration of immobility. No difference in locomotion was found between the groups, suggesting that the differences on depression-like behavior were not due to differences in locomotor activity. These findings illustrate that although estradiol decreases depression-like behavior (behavioral despair), it increases this behavior in adult mice given an immune challenge during puberty. These results suggest that exposure to an immune challenge during puberty alters the responsiveness of depression-like behavior to gonadal hormones.

Sources of Research Support: NIH NS19327 and an Isis grant from the Study of Women's Health Research.

Nothing to Disclose: NI, KMO, PHG, RR, SS, JDB

Pub # P3-234
Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Influences on Programming of Neuroendocrine Pathways (1:30 PM-3:30 PM)
Title Moderate Maternal Undernutrition Results in Epigenetic Changes in the Fetal Hypothalamic Glucocorticoid Receptor Gene
Author String G Begum, A Stevens, K Connor, J Challis, F Bloomfield, A White
University of Manchester, Manchester, UK; University of Toronto, Toronto, Canada; University of Auckland, Auckland, New Zealand

Body The hypothalamic glucocorticoid receptor (GR) modulates the appetite regulating neuropeptides, proopiomelanocortin (POMC) and neuropeptide Y (NPY). We have shown that these hypothalamic GR and POMC genes undergo significant epigenetic changes in fetal sheep following maternal undernourishment (1). Fetal twin sheep have altered physiology to singletons and are understood to be nutritionally challenged (2). Therefore we aim to determine if sheep twin fetuses have epigenetic changes in GR and POMC genes and whether maternal undernutrition alters this.

Ewes were undernourished for 60 days pre- and 30 days following mating (singletons and twins n=8), reducing maternal bodyweight by 10-15%, or given normal feed (singletons and twins n=8). Arcuate nucleus enriched hypothalami were dissected from fetuses at post-mortem on gestational days 131-135.

In the ventral hypothalamus, the GR promoter had significantly increased H3K9 acetylation (marker for transcriptional activation) in the singleton undernourished (40%), twin fed (53%) and twin undernourished (52%) groups, compared to the singleton control group. This was associated with decreased GR promoter methylation (55%, 52%, 65% respectively). In the singleton undernourished group, GR mRNA expression increased by 81%, compared to the singleton control and the twin fed and undernourished groups. Hypothalamic POMC H3K9 acetylation also increased in the singleton undernourished, twin fed and undernourished groups (44%, 43%, 40% increase respectively), which was associated with decreased POMC promoter methylation levels (63%, 60%, 73% decrease respectively). The decrease in promoter methylation for GR and POMC genes in the singleton undernourished, twin fed and twin undernourished groups was associated with a decrease in hypothalamic DNA methyltransferase activity (57%, 41% and 40% respectively). There were similar levels of POMC and NPY expression across all groups. Epigenetic changes in GR were not a generalised phenomenon as the control gene OCT4 showed no changes in epigenetic or expression status following maternal undernourishment.

The changes in epigenetic status of the GR gene in the hypothalamus of both twin groups were similar to the undernourished singleton group. This suggests that twinning is a programming event. Therefore twinning may cause epigenetic changes in hypothalamic GR, which may be linked to the increased likelihood of twins developing diabetes later in life.

(1) Stevens et al, *Endocrinology* 2010; 8:3652-3664

(2) Rumball et al, *J Physiol.* 2008;586(5):1399-411

Sources of Research Support: NIHR, Manchester Biomedical Research Centre.

Nothing to Disclose: GB, AS, KC, JC, FB, AW

Pub # P3-235
Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Influences on Programming of Neuroendocrine Pathways (1:30 PM-3:30 PM)

Title Maternally Activated Posterior Thalamic Neurons Possibly Convey the Suckling Information towards Hypothalamic Centers

Author String A Dobolyi, M Cservenak, TB Usdin, M Palkovits
Semmelweis University and the Hungarian Academy of Sciences, Budapest, Hungary; National Institute of Mental Health, Bethesda, MD

Body We recently identified a cell group in the posterior intralaminar thalamic nucleus (PIL), which is activated in mother rats. Besides Fos expression in response to pup exposure, a marked elevation in the immunoreactivity of tuberoinfundibular peptide 39 (TIP39) was also demonstrated in these neurons of rat dams. TIP39 fibers as well as the receptor for TIP39, the parathyroid hormone 2 receptor (PTH2 receptor), are present in a number of endocrine and limbic brain regions including the infralimbic cortex, several hypothalamic nuclei, the latera septal nucleus, and some amygdaloid nuclei (1). We showed that a recently developed antagonist of the PTH2 receptor dose-dependently inhibited the suckling induced prolactin release, probably *via* action in the hypothalamic arcuate nucleus (2). TIP39-containing fibers in limbic and hypothalamic brain centers could be originated in the PIL and also TIP39 neurons in 2 other groups present in the periventricular gray of the thalamus and the medial paralemnisal nucleus in the lateral pons, respectively. In the present study, we aimed at elucidating the projections of neurons in the posterior intralaminar thalamic nucleus. The anterograde tracer biotinylated dextran amine (BDA) was iontophoretically injected into the PIL of mother rats. The position of the injection site was histologically verified by double labeling the tracer with TIP39. We could follow the projections of PIL neurons to the preoptic area, the para-, periventricular, arcuate and dorsomedial nuclei of the hypothalamus, and in the medial and central nuclei of the amygdala where anterogradely labeled nerve terminals were located. In subsequent experiments, the retrograde tracer cholera toxin beta subunit (CTB) was injected into these target regions and confirmed that hypothalamic sites receive input from TIP39 neurons while the amygdala receives predominantly non-TIP39 input from the PIL. Based on data presented here we conclude that neurons in the PIL provide input to a number of hypothalamic and amygdala sites, which are in turn related to different aspects of maternal adaptations. In view of the ascending input to the PIL, the presence of maternally activated TIP39 neurons in the nucleus, and the recently established involvement of TIP39 in the regulation of suckling-induced prolactin release, we suggest that neurons in the PIL mediate or modulate hypothalamic effects of suckling and contribute to maternal adaptations.

- (1) Dobolyi A, Palkovits M, Usdin TB, The TIP39-PTH2 receptor system: unique peptidergic cell groups in the brainstem and their interactions with central regulatory mechanisms. *Prog Neurobiol* 2010; 90:29-59.
2) Cservenák M, Bodnár I, Usdin TB, Palkovits M, Nagy GM, Dobolyi A, Tuberoinfundibular peptide of 39 residues is activated during lactation and participates in the suckling-induced prolactin release. *Endocrinology* 2010; 151:5830-40.

Sources of Research Support: Bolyai Janos Award, Grants NKTH-OTKA K67646 and OTKA NNF85612 awarded to AD.

Nothing to Disclose: AD, MC, TBU, MP

Pub # P3-236

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Influences on Programming of Neuroendocrine Pathways (1:30 PM-3:30 PM)

Title Sexual Divergence in the Metabolic Effects of Testosterone

Author String SD Clarke, MA Cowley, IJ Clarke, BA Henry
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Body Adiposity is regulated in a sexually divergent manner. The sex differences are due, in part, to the differences in sex steroid profiles. Sex steroids are protective against obesity, with low levels increasing adiposity. Differential effects of androgens between the sexes are not well documented. We investigated effects of testosterone (T) on energy balance in male (n=6) and female sheep (n=4). Castrated animals (approx 55 kg) received 3x200mg T implants (s.c.) for 4 weeks or blank implants (controls). Dataloggers (SubCue) were implanted into retroperitoneal fat and skeletal muscle (hindlimb) to record temperature as an index of thermogenic output. Food was available between 1100-1600h daily, which entrains a post-prandial elevation in temperature. Food intake was measured. Prior to and after T-treatment, serial blood samples were taken (30min) between 0900-1600h to measure glucose, non-esterified fatty acids (NEFA), lactate and insulin. In males, a muscle biopsy was taken prior to feeding (0900h) and during feeding (1200h). A biopsy of fat tissue was collected at 1200h. Biopsies were used to measure levels of mRNA for uncoupling proteins (UCPs) and activation of AMP-activated protein kinase (AMPK) and Akt. T treatment did not change food intake in either sex. T treatment reduced adipose and muscle temperature in males only, without affecting post-prandial temperature. In males, pre-prandial levels of glucose were higher but NEFA levels were lower than in females. The post-prandial elevation in glucose was reduced in T-treated males (vs control), but not in females. There was no effect of T on lactate or insulin levels. In males, post-prandial expression of UCP 1 and UCP3 mRNA was higher in skeletal muscle than in the pre-prandial period and this effect was irrespective of T status. T had no effect on UCP 1, 2 or 3 mRNA in skeletal muscle or adipose tissue in castrate rams. Nor did T treatment change the phosphorylation of AMPK or Akt (ser 473, thr 308) in skeletal muscle of rams. Thus, T impacted on metabolic state of male but not female sheep. In males, T reduced temperature in fat and muscle tissues, without an effect on thermogenesis. Furthermore, in males, T reduced the post-prandial glucose levels, without altering food intake or AMPK and Akt signalling in muscle. The mechanism underlying this sex-specific effect of T is not known but is likely to be due to sexual differentiation of the brain centres controlling energy expenditure.

Nothing to Disclose: SDC, MAC, IJC, BAH

Pub # P3-237

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Influences on Programming of Neuroendocrine Pathways (1:30 PM-3:30 PM)

Title Hippocampal Aromatase Expression in Alzheimer Disease

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Body Postmenopausal women are at a higher risk of developing Alzheimer's disease (AD) as compared to men of the same age. This increased risk is presumably linked to hormonal changes during menopause. Although numerous experiments have demonstrated that the steroid hormone estradiol (E2) has neuroprotective characteristics relevant to AD (i.e. reduction of β -amyloid accumulation), results of clinical studies testing the effectiveness of hormone replacement as a potential AD therapy are inconclusive. This ambiguity may be due to the fact that most studies did not take into consideration that the brain itself is a source of steroid hormones including E2. In the hippocampus, a brain region that is severely affected in AD, locally synthesized E2 regulates synaptic plasticity, neuronal cell death, and axonal outgrowth, all of which are affected by AD. Here, we asked for the first time whether hippocampal expression of aromatase, the enzyme that converts testosterone into E2, is altered in AD.

We used human hippocampal tissue obtained post-mortem from patients diagnosed with AD to perform aromatase immunostaining followed by computer based image analysis. Immunoreactivity was significantly higher in the samples from AD patients as in samples from age- and sex-matched control patients, who showed no signs of neurodegenerative disease. Due to inherent difficulties with human post-mortem tissue we used transgenic mice for more detailed studies. 5XFAD mice express high levels of β -amyloid, and at 3 months they develop symptoms of AD. mRNA expression of total aromatase and of the gonad-specific first exon of aromatase were detected by quantitative RT-PCR in hippocampi of male and female 5XFAD mice at the age of 3, 6, and 12 months. Expression levels were compared with those of age-matched wild-type (WT) mice. Total aromatase levels did not differ at any time point, however, a significant decrease in the expression of the gonad-specific first exon mRNA was detected in the 12 months old 5XFAD mice as compared to the WT mice. Comparison of aromatase protein expression by immunohistochemistry in conjunction with computerized image analysis revealed ~2-fold stronger aromatase immunoreactivity in the hippocampus of 12 months old 5XFAD mice as compared to the WT animals. Further experiments will be carried out to test whether the observed increase in hippocampal aromatase expression in AD is induced by a pathological event in AD and whether it is a protective mechanism.

Nothing to Disclose: DAD, DAG, GMR, JP-K

Pub # P3-238

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Influences on Programming of Neuroendocrine Pathways (1:30 PM-3:30 PM)

Title Neuroprotective and Immunoregulatory Properties of Vitamin D in a Stroke Model

Author String R Balden, A Selvamani, F Sohrabji
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Body The risk and severity of stroke increases with age and may be associated with the age-related decline in factors that regulate vascular and immune function such as estrogen, Vitamin D, IGF-1. In the present studies we specifically determined whether age related changes in Vitamin D were associated with infarct severity, and Vitamin D's neuroprotective role in stroke in animals that were (a) deprived of Vitamin D in diet or (b) administered Vitamin D post stroke. Vitamin D levels measured in plasma of adult (6-7 m old) and middle aged (10-12 m) Sprague-Dawley rats were found to be lower in middle-aged animals, and were inversely correlated with the severity of stroke infarct. To directly assess the neuroprotective role of vitamin D, adult female rats were fed control or Vitamin D deficient (def) diet for 8 weeks and subject to middle cerebral artery occlusion (stroke) thereafter. Vitamin D-def diet reduced circulating levels to 22% of that observed in animals fed control chow. Furthermore, Vitamin D-def animals had significantly larger cortical and striatal infarct volumes as compared to control chow animals. Analysis of cytokine expression in brain tissue revealed that D-deficient animals also expressed significantly less IL-1 α , IL-1 β , IL-2, IL-4, and IFN- γ in the ischemic cortex, and less IL-1 α and IL-10 in the ischemic striatum ($p < .05$). However, ischemia-induced expression of IL-6 was significantly elevated in Vitamin D deficient animals. Furthermore, IL-6 expression correlated significantly with infarct volume ($r^2 = .83$ cortex, $r^2 = .90$ striatum). A separate study on post-stroke administration of Vitamin D to stroke-affected animals found no improvement in Vitamin D treated animals. Our data indicate that chronic Vitamin D deficiency can exacerbate stroke-induced cell loss, possibly due to chronic physiological alterations in the immune system. Acute Vitamin D replacement, which did not regulate the same complement of cytokines, was not found to be neuroprotective.

Sources of Research Support: Supported by NIH Grants AG027684 and AG028303 to FS.

Nothing to Disclose: RB, AS, FS

Pub # P3-239

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Influences on Programming of Neuroendocrine Pathways (1:30 PM-3:30 PM)

Title Regulation of TORC1 and Its Role in the Adrenergic Induction of AA-NAT in Rat Pinealocytes

Author String N Amyotte, M Ferguson, R Kanyo, CL Chik, AK Ho
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Body Salt-inducible kinase 1 (SIK1) has recently been shown to repress the adrenergic induction of arylalkylamine-N-acetyltransferase (*Aanat*), a cAMP response element-binding protein (CREB)-target gene in the rat pineal gland. Whether this effect of SIK1 is mediated through its interaction with the transcription co-activator, transducer of regulated CREB activity (TORC) remains to be determined. In this study, we investigated the regulation of TORC1 in the rat pineal gland and its role in modulating AA-NAT induction. Whole gland study demonstrates that TORC1 is tightly regulated in the rat pineal gland. This regulation is seen on Western blots as a rapid band mobility shift immediately after the onset of darkness. In cultured pineal cells, NE stimulation induces a similar regulatory pattern of TORC1 as observed in the nocturnal pineal gland and this occurs in the absence of an effect on the mRNA level of *Torc1*. Dephosphorylation is involved in the NE-mediated mobility shift of TORC1. Characterization of the transduction pathway indicates the involvement of alpha-adrenergic receptors through elevation of intracellular calcium with phosphoprotein phosphatase 2B (PP2B) being the phosphatase involved. Nuclear/cytosolic fractionation of cultured pineal cells reveals that the same signalling pathway and PP2B are responsible for translocating dephosphorylated TORC1 into the nucleus, where TORC1 is presumed to be active. To investigate the relationship between SIK1 and TORC1, depleting endogenous SIK1 using silencing RNA allows TORC1 to remain in the nucleus longer while there is a corresponding reduction in cytosolic TORC1. A modulatory role of TORC1 in NE-mediated AA-NAT induction is supported by the enhancing effect on NE-induction of *Aanat* expression in pinealocytes over-expressing TORC1. Taken together, our results indicate that TORC1 is regulated by an adrenergic-mediated calcium-dependent mechanism in the rat pineal gland and PP2B is involved in translocating dephosphorylated TORC1 into the nucleus. Moreover, the TORC1/SIK1 pathway is involved in the NE induction of AA-NAT induction.

Nothing to Disclose: NA, MF, RK, CLC, AKH

Pub # P3-240

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Influences on Programming of Neuroendocrine Pathways (1:30 PM-3:30 PM)

Title Regulation of Transducer of Regulated cAMP-Response Element Binding-Protein Activity 2 (TORC2) in Rat Pinealocytes

Author String R Kanyo, N Amyotte, CL Chik, AK Ho
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Body Transducer of regulated cAMP-response element binding-protein (CREB) activity 2 (TORC2), a co-activator of CREB, has previously been shown to regulate CREB-target genes, and therefore may play a role in the beta-adrenergic / protein kinase A / CREB-mediated gene transcription in the rat pineal gland. Although *Torc2* mRNA is expressed in the rat pineal gland, there is no induction by norepinephrine (NE). However, adenovirus-mediated over-expression of *Torc2* in pinealocytes results in an increase in NE-stimulated gene transcription. Here we report a NE-mediated dephosphorylation, nuclear translocation, and degradation of TORC2 in cultured rat pineal cells. Western blot analysis reveals that treatment of pinealocytes with NE results in a reduction in the lower MW band of TORC2. Experiments with calf intestinal phosphatase and cytoplasm/nuclear fractionation indicate that the lower MW band represents dephosphorylated TORC2. The NE-mediated TORC2 dephosphorylation and nuclear accumulation is blocked by co-treatment with propranolol, a beta-adrenergic antagonist, but not by prazosin, an alpha1-adrenergic antagonist, and mimicked by dibutyryl cAMP, but not by other kinase activators. Moreover, the NE-mediated TORC2 dephosphorylation is blocked by okadaic acid and calyculin A, two phosphoprotein phosphatase (PP) inhibitors with potent inhibitory effects on PP2A, but not by tautomycin, a potent inhibitor against PP1A or cyclosporine A, an inhibitor of PP2B. Together, our results indicate that the NE-mediated dephosphorylation, nuclear translocation and degradation of TORC2 is mediated by the beta-adrenergic receptor/protein kinase A pathway in rat pinealocytes with PP2A being the phosphatase involved.

Nothing to Disclose: RK, NA, CLC, AKH

Pub # P3-241

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Influences on Programming of Neuroendocrine Pathways (1:30 PM-3:30 PM)

Title The Brain-Derived Neurotrophic Factor (*BDNF*) SNP Rs12291186 Minor C Allele Is Positively Associated with BMI, Negatively Associated with *BDNF* Expression in Human Ventromedial Hypothalamus (VMH), and Alters Yin-Yang 1 (YY1) Transcription Factor Binding

Author String Z Mou, E Morgun, TM Hyde, BK Lipska, C-J Ong, LJ Marshall, AH Ali, EA Stern, JW Tsao, JE Kleinman, JA Yanovski, JC Han
Eunice Kennedy Shriver NICHD, Bethesda, MD; GCAP, IRP, NIMH, Bethesda, MD; USUHS, Bethesda, MD; NINDS, Bethesda, MD

Body
Background: Evidence suggests BDNF is a downstream anorexic mediator of CNS leptin action, playing an important role in regulating energy homeostasis. Allelic variations in single nucleotide polymorphisms (SNPs) within the *BDNF* gene locus previously have been associated with alterations in BMI, but the mechanisms by which such SNPs might alter brain *BDNF* expression have not been established. We hypothesized that *BDNF* SNPs that increase transcriptional repressor binding would inhibit *BDNF* expression and thus promote obesity.
Methods: Genomatix MatInspector software identified that the minor C allele of *BDNF* SNP rs12291186 forms a potential repressor binding site for the YY1 transcription factor. We genotyped rs12291186 in 472 healthy adults (40.7±1.6y, 67%F, 36% Black, BMI 32.7±7.9kg/m²) and 575 healthy children (12.1±3.3y, 55%F, 39% Black, BMI-Z 1.29±1.21) and assessed *BDNF* expression in VMH region mRNA from 86 adults with sudden death (age 40.7±13.6y, 34%F, 62% Black, BMI 32.6±11.3kg/m², postmortem interval [PMI] 31±15h, RNA integrity number [RIN] 8.0±0.8). ANCOVAs compared BMI (adjusted for age, sex, race), BMI-Z (adjusted for race), and VMH *BDNF* expression (adjusted for age, sex, race, PMI, and RIN) by genotype. Electrophoretic mobility shift assays (EMSAs) were performed to determine if YY1 binding to oligonucleotide probes representing the rs12291186 major A allele sequence differed from binding to the minor C allele sequence.
Results: rs12291186 was associated with BMI in adults (AA vs. AC vs. CC, adjusted mean±SEM: 31.3±1.0 vs. 33.7±1.0 vs. 35.6±1.0 kg/m², p=0.005), BMI-Z in children (1.21±0.06 vs. 1.41±0.11 vs. 1.79±0.21, p=0.02), and relative VMH *BDNF-transcript-IIb* expression (1.0±0.1 vs. 1.3±0.1 vs. 0.6±0.1, p=0.046). EMSAs demonstrated a marked increase in protein binding to the C allele probe when incubated with nuclear extract compared to the A allele probe. Competition assays using unlabelled A allele and C allele probes confirmed higher binding affinity of protein to the C allele. Addition of either anti-YY1 antibody or unlabelled probes bearing established sequences for YY1 binding sites decreased the intensity of the initial DNA-protein complexes, suggesting specificity of binding to YY1.
Conclusions: Homozygosity for the minor C allele at rs12291186 is associated with higher BMI in adults, higher BMI-Z in children, and lower VMH *BDNF* expression, possibly due to increased binding affinity for YY1 leading to repression of BDNF transcription.

Sources of Research Support: Intramural programs of the Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institute of Mental Health, and the National Institutes of Health Clinical Center Bench-to-Bedside Award Program.

Nothing to Disclose: ZM, EM, TMH, BKL, C-JO, LJM, AHA, EAS, JWT, JEK, JAY, JCH

Pub # P3-242

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Influences on Programming of Neuroendocrine Pathways (1:30 PM-3:30 PM)

Title Cerebrospinal Fluid (CSF) Levels of Proopiomelanocortin (POMC) and Agouti-Related Protein (AgRP) in Human Pregnancy: Evidence for Leptin Resistance

Author String G Page-Wilson, E Reitman-Ivashkov, K Meece, A White, M Rosenbaum, RM Smiley, SL Wardlaw
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Body Pregnancy is characterized by elevated plasma leptin levels in both animals and humans. Leptin decreases food intake by modulating the expression of hypothalamic neuropeptides, including POMC and AgRP. The POMC-derived MSH peptides inhibit food intake while AgRP stimulates food intake by antagonizing effects of MSH at brain melanocortin receptors. Yet, in spite of the hyperleptinemia of pregnancy, appetite and caloric consumption increase. In order to investigate leptin transport and levels of target neuropeptides in human pregnancy we measured leptin, POMC, and AgRP in CSF and plasma leptin in samples obtained from 21 fasting pregnant women prior to caesarean section and from 13 fasting non-pregnant women in the follicular phase of the menstrual cycle. Plasma leptin was measured by RIA and CSF leptin and AgRP were measured using highly sensitive ELISAs. POMC was measured using a specific monoclonal based ELISA that detects the intact POMC prohormone that we have previously shown to be the predominant POMC peptide in CSF. BMI was 31.3 ± 1.3 (SEM) vs 26.8 ± 1.7 in pregnancy vs controls, while pre-pregnancy BMI was comparable to controls (24.6 ± 1.1). Consistent with previous findings, plasma leptin levels were significantly higher in pregnant women (32.8 ± 4.6 vs 17.5 ± 3.2 ng/ml, $p=0.02$). In spite of the nearly two-fold elevation in plasma leptin in pregnant subjects, mean CSF leptin did not differ between the two groups (283 ± 34 vs 311 ± 32 pg/ml), consistent with a relative decrease in leptin transport into CSF during pregnancy. Accordingly, the CSF/plasma leptin percentage was $1.0 \pm 0.01\%$ in pregnant subjects vs $2.1 \pm 0.2\%$ in controls ($p < 0.0001$). Mean CSF levels of the orexigenic peptide AgRP were significantly higher in pregnant subjects (32.4 ± 2.7 vs 24.1 ± 2.6 pg/ml, $p=0.02$). Mean CSF levels of the anorexigenic peptide precursor POMC tended to be lower in pregnant subjects (200 ± 14 vs 225 ± 18 fmol/ml), but this difference was not significant. However, the POMC/AgRP ratio was significantly lower among pregnant women (6.8 ± 0.6 vs 10.1 ± 0.9 , $p=0.004$), consistent with an overall decrease in melanocortin tone favoring increased food intake during pregnancy. These data demonstrate that during pregnancy women are protected from the suppressive effects of hyperleptinemia on appetite by both decreased leptin transport into brain and by resistance to leptin's effects on target neuropeptides that regulate energy balance.

Nothing to Disclose: GP-W, ER-I, KM, AW, MR, RMS, SLW

Pub # P3-243

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Influences on Programming of Neuroendocrine Pathways (1:30 PM-3:30 PM)

Title Identification of a New Gene Involved in IHH and Ataxia by Positional Cloning in a Patient with a Balanced Translocation t(3;12)(p13;p13)

Author String H-G Kim, R Ullmann, H-H Ropers, V Kalscheuer, L Layman
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Body Patients with idiopathic hypogonadotropic hypogonadism (IHH) present with absent puberty due to a hypothalamic-pituitary defect, and may be either normosmic (nIHH) or anosmic -Kallmann syndrome (KS). Although mutations in genes such as FGFR1, KAL1, CHD7, PROKR2, and GNRHR constitute the most commonly encountered etiologic genes in IHH/KS, the molecular basis for most patients remains unknown. Approximately 1/2,000 liveborns possess a de novo balanced translocation, and about 6% of these patients manifest the phenotype of a significant, developmental disorder. These rare, but exceedingly informative patients provide the unique opportunity to identify new genes causing birth defects. We were able to ascertain a male patient with a mos46,XY,t(3;12)(p13;p13)[18]/46,XY[3] with nIHH and cerebellar ataxia, which affords the opportunity to identify a gene important in pubertal development. Array Comparative Genome Hybridization (CGH) analysis of patient DNA was consistent with a balanced translocation and excluded Copy Number Variation (CNV) as the cause of the IHH phenotype. In this patient with the mosaic 3;12 translocation, homogeneous lymphoblastoid cell lines with the balanced translocation were successfully transformed from peripheral white blood cells. IHH and cerebellar ataxia often occur together and they are seen in Gordon Holms syndrome and Boucher-Neuhauser syndrome. We hypothesize that one of the breakpoints of this translocation case is likely to harbor a gene responsible for this phenotype. A positional cloning technique was applied to clone each of the breakpoints. FISH mapping for a breakpoint at 12q13 and array painting in this patient is underway. This chromosome translocation affords the potential to identify a gene involved in IHH and ataxia.

Nothing to Disclose: H-GK, RU, H-HR, VK, LL

Pub # P3-244

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Influences on Programming of Neuroendocrine Pathways (1:30 PM-3:30 PM)

Title Cell Replication and Angiogenesis in Central Nervous System Tumors and Their Relationship with the Expression of Tissue Prolactin and Hyperprolactinemia

Author String DMD Abech, JFS Pereira-Lima, CGS Leaes, RT Meurer, LM Barbosa-Coutinho, NP Ferreira, MdC Oliveira UFCSPA, Porto Alegre, Brazil

Body Introduction: Prolactin (PRL) has mitogenic, antiapoptotic, morphogenic, secretory and angiogenesis modulation effects. The expression of PRL and its receptor has been described in breast and prostate tumor tissues, and more recently in central nervous system tumors. Objective: To assessed the effect of intracellular PRL (ICPRL) and hyperprolactinemia on cell replication, using an immunohistochemical (IHC) technique for Ki-67 and Mcm-2, and angiogenesis, using IHC for endoglin CD-105, in central nervous system tumors (CNS) of neuroepithelial tissue and meningiomas. Patients and Methods: This cross-sectional study included 79 cases of primary CNS tumors of neuroepithelial (41.8% of all cases: 10.2% astrocytomas, 24% glioblastomas and 7.6% oligodendrogliomas) and meningeal (58.2%) origin that were surgically excised at Hospital São José, Santa Casa de Porto Alegre, Brazil, over a period of 40 months. Patient age ranged from 1 to 86 years (mean 55.6 years) and 67% were women. This study was approved by the Research Ethics Committee of the Federal University of Health Sciences of Porto Alegre (UFCSPA), all patients signed an informed consent form, and the authors signed a term of confidentiality. All patients were operated on by the same neurosurgeon (N.P.F.). The streptavidin-biotin-peroxidase method was used to detect Ki-67, Mcm-2, ICPRL and endoglin (CD-105). Results: The medians for Ki-67 and Mcm-2 indexes, calculated as a percentage of marked cells, were significantly lower in meningiomas than in glioblastomas ($p < 0.001$ for Ki-67 and $p < 0.001$ for Mcm2) and oligodendrogliomas ($p < 0.001$ for Ki-67 and $p = 0.02$ for Mcm2). A good correlation was observed between the Ki-67 and Mcm-2 ($r_s = 0.60$) replication markers. There were no significant differences in vascular density between the different histological types. Immunohistochemistry for ICPRL was positive in 45.6% of the tumors. Serum PRL was elevated in 30,6% of the cases. Multiple regression analysis did not reveal any important correlation of ICPRL and serum PRL on Ki-67 and Mcm-2 indexes or vascular density. The analysis of the combined impact of ICPRL and serum PRL variables revealed a trend towards an increase in microvessel density in tumor tissue and a significant increase in cell replication markers ($p = 0.009$ for Ki-67 and $p = 0.05$ for Mcm-2). Conclusion: PRL in tumor tissue may be a modulator factor of cell proliferation in the central nervous system.

Nothing to Disclose: DMDA, JFSP-L, CGSL, RTM, LMB-C, NPF, MdCO

Pub # P3-245
Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Influences on Programming of Neuroendocrine Pathways (1:30 PM-3:30 PM)
Title Influence of Rest Interval Manipulation on Acute Testosterone and Cortisol Responses to Two Volume-Load Controlled Resistance Training Protocols in Healthy Men
Author String MG Villanueva, MG Villanueva, T Schroeder
University of Southern California, Los Angeles, CA; California State University, Fresno, CA

Body **Background:** Resistance training (RT) is a potent stimulator of skeletal muscle growth, and androgen signaling is important for mediating RT-induced muscle growth. While evidence suggests testosterone (T) promotes protein synthesis and possesses anti-catabolic properties within skeletal muscle, the catabolic hormone cortisol (C) alters the balance between hormone-mediated anabolic and catabolic activity.
Purpose: To examine the acute total testosterone (TT) and C responses to 4 RT protocols in healthy men. We hypothesized that hypertrophic (H) and strength (S) RT protocols equated for volume-load (sets x repetitions : load) and employing short rest intervals (RI) between sets will elicit acute increases in TT and C.
Methods: 6 men, 26±2.4 years of age, visited the laboratory for 1-repetition maximum (1-RM) strength testing and 4 randomized total body RT sessions that consisted of 4 exercises. In the S protocol, 8 sets of 3 repetitions were performed at 85% 1-RM for all exercises. In the H protocol, 3 sets of 10 repetitions were performed at 70% 1-RM for all exercises. The RI utilized: 60 seconds (S60, H60) and 90 seconds (S90, H90). Blood was drawn pre- (PRE), immediately post- (IP), 15 minutes post- (+15), and 30 minutes post-exercise (+30). TT and C concentrations were measured by ELISA. Paired samples t-tests were performed, with significance set at p<0.05.
Results: H60 elicited significant increases in TT concentrations from PRE (7.32±1.85 ng/mL) to IP (8.87±1.83 ng/mL), +15 (8.58±2.15 ng/mL), and +30 (8.28±2.16 ng/mL). H90 did not elicit significant changes in TT concentrations from pre- to any time point post-exercise. S60 showed a notable non-significant (p = 0.056) difference in TT concentration from PRE (7.73±1.88 ng/mL) to +15 (8.35±1.64 ng/mL), and S90 showed a noticeable non-significant (p = 0.07) difference in TT concentration from PRE (7.96±2.29 ng/mL) to IP (8.75±2.45 ng/mL). All four acute protocols did not significantly increase C concentrations from pre- to any time point post-exercise (p>0.05).
Conclusions: Utilizing short rest intervals in between RT sets maximize the acute TT response to hypertrophic schemes, potentially optimize the acute TT response to strength schemes, and do not significantly increase acute C concentrations. This may lead to simultaneous enhancements in both muscle strength and size over a longer-term period of RT, by modulating anabolic and catabolic processes that favor tissue growth over tissue breakdown.

Nothing to Disclose: MG, MG, TS

Pub # P3-246

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Influences on Programming of Neuroendocrine Pathways (1:30 PM-3:30 PM)

Title Parametric Imaging Analyzes Neuroendocrine Hormones and Dopamine Type 2 Receptor Availability

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Body Rodent studies indicate that mesolimbic and nigrostriatal dopamine (DA) neurotransmission is modulated by hormones, insulin, leptin and ghrelin. The levels and signaling of these hormones are altered in obesity and hypothesized to contribute to deficits in brain DA signaling. Parametric imaging analyses was used to assess the relationships between hormones levels and DA type 2 receptor (D2R) availability estimated by positron emission tomography (PET) imaging with [¹⁸F] fallypride. All subjects underwent MRI of brain. After an 8 h fast, plasma for hormones was collected and subjects underwent PET with [¹⁸F] fallypride to determine DA D2R binding potential (BP_{ND}). Serial PET scans were co-registered to each other and MRI scans. Parametric images of DA D2R were co-registered across all subjects with an elastic deformation algorithm. Correlations of hormones with parametric DA D2R images were calculated voxel by voxel (4x4x4 mm) with Pearson product moment correlation. Clusters were delineated with a cutoff of $p < 0.01$ for voxel and cluster (minimal 21 voxels). Clusters with < 21 had a cut off of $p < 0.05$ unless small volume correction was completed allowing for of $p < 0.01$. T-tests were used for between-group comparisons and presented as mean \pm SD, and lean vs obese, respectively. Subjects included, 22 females, 8 lean (BMI= 22 \pm 3 kg/m²) and 14 obese (BMI = 38 \pm 5, kg/m²), who were similar in age(41 \pm 9 vs 40 \pm 8yrs, $p=0.697$). Insulin (6 \pm 2 vs 19 \pm 12, $p=0.001$) and leptin (12 \pm 6 vs 45 \pm 12, $p<0.001$) were higher in obese, while acyl ghrelin was lower (253 \pm 93 vs 105 \pm 57 $p<0.001$). Acyl ghrelin had negative relationships with DA D2R including clusters involving the inferior temporal cortex, temporal pole, and insular cortex on the right (458 voxels, $r= -0.58$) and left (452 voxels, $r=-0.54$). Also relationships with acyl ghrelin included clusters involving the ventral striatum extending into ventral caudate and putamen on the right (45 voxels, $r=-0.56$) and left (52 voxels, $r=-0.56$). Leptin levels correlated with clusters (16 voxels, $r=0.65$) in the region of the hypothalamus, left ventral striatum and caudate (27 voxels, $r=0.60$), and temporal cortex and collateral sulcus on the right (75 voxels, $r=0.64$) and left (38 voxels, $r=0.63$). Insulin correlated with 2 clusters involving the dorsal medial thalamus (34 voxels, $r=0.67$) and right superior cortex (31 voxels, $r=0.63$). Parametric image analyses reveal noteworthy relationships between neuroendocrine hormones and DA D2R availability.

Sources of Research Support: NIEHS K12 ESO15855 (J.P.D.), NIDDK RO1-DK070860, (N.N.A.), VU CTSA (1 UL1 RR024975), VU DRTC (DK20593).

Nothing to Disclose: JPD, RL, MSA, PM-S, JK, NNA, RK

Pub # P3-247

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Neuroendocrine Tumors (1:30 PM-3:30 PM)

Title HSP90 Inhibitors: a Novel Therapeutic Approach for the Treatment of Neuroendocrine Tumors

Author String G Ailer, K Zitzmann, G Vlotides, G Spoettl, B Goeke, CJ Auernhammer
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Body The HSP90 chaperone machine involved in numerous oncogenic signaling pathways is overexpressed in cancer cells and is currently being evaluated for anticancer therapy. Neuroendocrine tumors (NETs) of the gastroenteropancreatic (GEP) system comprise a heterogenous group of tumors with increasing incidence and poor prognosis. Here we report antiproliferative effects of the HSP90 inhibitors AU922 and HSP990 (Novartis) in neuroendocrine tumor cells. Treatment of human pancreatic BON1, bronchopulmonary NCI-H727 and midgut carcinoid GOT1 neuroendocrine tumor cells with increasing concentrations of AU922 and HSP990 dose-dependently suppressed cell proliferation. Significant effects on neuroendocrine cell proliferation were observed with inhibitor concentrations as low as 5 nM. Inhibition of cell proliferation was associated with induction of apoptosis, demonstrated by increased number of cells in sub-G0/1 phase of the cell cycle as well as induction of PARP cleavage in BON1, NCI-H727 and GOT1 cells. HSP90 inhibition was associated with decreased neuroendocrine ERK, Akt and GSK1 phosphorylation and induction of HSP70 expression. Targeted inhibition of upregulated HSP90 activity could be useful for the treatment of aggressive neuroendocrine tumors resistant to conventional therapy.

Nothing to Disclose: GA, KZ, GV, GS, BG, CJA

Pub # P3-248

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Neuroendocrine Tumors (1:30 PM-3:30 PM)

Title The Inhibitor of Cap-Dependent Translation 4E-BP1 as a Crossroad between Somatostatin Analogs and Everolimus: Mechanisms and Consequences on Combined Antitumoral Action of Both Drugs

Author String S Najib, L Guitton-Sert, C Marboeuf, N Saint-Laurent, D Fourmy, HA Schmid, C Susini, S Pyronnet, C Bousquet
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Body Somatostatins have a broad range of biological actions including inhibition of exocrine and endocrine secretions but also of cell proliferation and survival, acting *via* G protein-coupled somatostatin receptors (sst_s, 1-5). Sst_s are widely expressed in normal and tumor cells. Sst2 is the most frequently expressed subtype in tumor cells and synthetic somatostatin analogs which activate mainly sst2 are clinically used for the diagnosis and treatment of patients with endocrine tumors.

Our recently published data demonstrate that the molecular mechanisms underlying sst2 antitumoral action include the inhibition of the survival phosphatidylinositol 3-kinase (PI3K). This involves a physical and ligand-regulated interaction between sst2 with the PI3K regulatory p85: upon somatostatin analog treatment, a pre-existing complex comprising sst2 and p85, which we showed to account for a significant cell basal PI3K activity, is disrupted thereby inhibiting PI3K activity. We now identify the scaffolding protein filamin-A (FLNA) as a critical player regulating the dynamic of this molecular complex in 3 different cell lines. Upon cell-treatment with somatostatin analog, FLNA competes with p85 for binding to sst2, thereby forcing the disruption of the pre-existing sst2-p85 complex and inhibiting PI3K activity. Knocking-down FLNA expression or mutating the FLNA-binding site on sst2, identified by molecular modeling of the sst2-p85-FLNA complex, reversed sst2 inhibitory role on PI3K and antitumoral signaling.

Interestingly, recent therapeutic trials have demonstrated that the combination of somatostatin analogs and everolimus, an inhibitor of mTOR, offers a promising antitumoral treatment option for endocrine tumors. We here describe a possible scientific rationale for the combined use of both drugs on inhibition of pancreatic cancer AR4-2J cell survival, which is inhibited by 39 ± 6 % with 10⁻⁷ M SOM230, by 66 ± 10 % with 10⁻⁷ M RAD001 and by 89 ± 9 % with combined treatment. Indeed, an interesting PI3K target of sst2 includes the inhibitor of the cap-dependent translation 4E-BP1 whose expression is induced upon somatostatin analog treatment. As everolimus also activates 4E-BP1 by preventing its phosphorylation by mTOR, we demonstrate that, in tumors treated concomitantly with somatostatin analog (SOM230) and everolimus (RAD001), only the active hypophosphorylated forms of 4E-BP1 accumulates, thereby increasing the inhibitory effect of 4E-BP1 on tumor growth.

Nothing to Disclose: SN, LG-S, CM, NS-L, DF, HAS, CS, SP, CB

Pub # P3-249

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Neuroendocrine Tumors (1:30 PM-3:30 PM)

Title Metastatic Digestive Neuroendocrine Tumors: The Role of Hepatic Arterial Embolization/Chemoembolization

Author String J Couto, AP Santos, RG Martins, MJ Sousa, MJ Bento, I Torres
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Body

Introduction: Up to 60% of patients with neuroendocrine tumors also have liver metastases (LM) leading to worse survival rate. Ablative therapies (AT) such as selective hepatic trans-catheter arterial embolization (TAE) or chemoembolization (TACE) can reduce tumor size and hormonal secretion being effective in the palliation of symptoms. Previous studies reported a median survival after embolization ranging from 13-80 months (mos.).

Methods/design: Patients with metastatic digestive neuroendocrine tumours (NETs) who underwent TAE or TACE, between January 2005 and November 2010, were included in this retrospective study. Gender, localization of primitive lesion, OMS classification, treatment and survival after treatment were some of the evaluated parameters. Statistical analysis was performed using SPSS, version 17.0. Overall survival (OS) was calculated by Kaplan-Meier method and comparisons within variables were made using Breslow and Long Rank tests.

Results: This study included 19 patients (pts.), mean age: 54.6±12.1y; 42%M / 58%F; 68% pts. with Gastrointestinal (GINETs), 32% with Pancreatic NETs (PETs); 21% G1 and 79% G2; 16% with simple pattern, 5% with complex pattern and 79% with diffuse pattern of LM; 21% of pts. treated with TACE and 79% of pts. treated with TAE. Median OS for the entire group of patients was 25 mos.; 21 for M and 26 for F (P = 0.34). OS was 25 mos. for both GINETs and PETs (P=0.68). OS doesn't seem to correlate with age (25 vs. 26 mos.; pts [le]52 vs. >52y; P=0.43). All of G1pts. vs. 46,7% G2 pts. were alive at last evaluation.

Conclusion: This study was limited by its retrospective nature and by the small number of patients included. Nevertheless, concerning the effect of AT on survival, characteristics like gender, age, and primary localization of NETs didn't change the outcomes. G1 pts had better survival, although it is necessary to compare with pts. with the same grade and no previous AT to conclude if this therapy alters the outcome of the disease.

Nothing to Disclose: JC, APS, RGM, MJS, MJB, IT

Pub # P3-250

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Neuroendocrine Tumors (1:30 PM-3:30 PM)

Title Expression of Somatostatin Receptors and Dopamine 2 Receptor in Lung Carcinoids as a Possible Biotherapy Target

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Body **INTRODUCTION:** Expression of somatostatin receptors (SSTRs) and dopamine 2 receptor (D2R) in neuroendocrine tumours is of clinical importance, as somatostatin analogues and dopamine agonists are used in biotherapy.
AIMS: To examine the expression of the five SSTR subtypes and D2R in lung carcinoids (LCs).
MATERIALS & METHODS: Tumour specimens from 87 patients with primary LCs were included and clinical data regarding sex, age at diagnosis and size of the primary tumour were recorded. Tumours were classified in Typical and Atypical according to the WHO 2004 criteria. The expression of all SSTR subtypes and D2R was evaluated immunohistochemically, and statistically correlated to tumour type. The tumours were considered as positive if receptor immunoreactivity appeared in the majority of the tumour cells.
RESULTS: Among 87 patients 50 (58%) were female and the mean age at diagnosis was $52,5 \pm 15,7$ years. Seventy three tumours were Typical and 14 Atypical. The mean diameter of the primary tumour was $24,4 \pm 12$ mm. SSTR2 was the receptor type most frequently expressed (71%), followed by SSTR1 (68%), SSTR5 (31%) and SSTR3 (11,5%). SSTR4 was not detected in any of the tumours. D2R was seen in 72% of the tumours and co-expressed with SSTR2 in 58%. The expression of the above receptors was not correlated to the histological type of LCs or the size of the primary tumour.
CONCLUSIONS: The high relative incidence of SSTR1, SSTR2 and in a lesser extent of SSTR5 and SSTR3 makes promising the biotherapy of LCs with universal SSTR agonists. Moreover, the co-expression of SSTR2 and D2R in a significant number of these tumours favours also the use of novel chimeric compounds in their management.

Sources of Research Support: Hellenic Endocrine Society.

Disclosures: GK: Coinvestigator, Novartis Pharmaceuticals. Nothing to Disclose: LG, RT, GK, AT

Pub # P3-251

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Neuroendocrine Tumors (1:30 PM-3:30 PM)

Title Does Pancreatic Polypeptide Help in the Diagnosis of Pancreatic Endocrine Tumors in MEN-1 Syndrome?

Author String LP Garcia-Vagas, G Lastra
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Body

Background:
Screening studies have revealed that symptomatic pancreatic endocrine tumors (PET) occur late in MEN-1 and when a clinical syndrome of hormone excess is awaited, up to 50% of patients already have metastasis (1). Pancreatic polypeptide (PP) was considered a screening test for PET but its use declines because its low sensitivity (2), however a PP value 3 times above the normal limit, has a sensitivity and specificity to identify radiographically PETs of 95 and 85% respectively (3).

Case:
36 year old female with family Hx of MEN-1 syndrome genetically confirmed in her kindred, presented with recurrent urolithiasis. She was otherwise asymptomatic. Physical exam was unremarkable. A serum calcium of 10.7mg/dl (8.4-10.5mg/dl), PTH 133 pg/ml (15-75 pg/ml) and 24 hour urine calcium 408 mg(50-250mg/24 hours) made the diagnosis of primary hyperparathyroidism. She underwent surgical removal of 3 1/2 parathyroid glands with cryopreservation. Other tests are reported below: prolactin 24 ng/ml(5-23ng/ml), gastrin 98 pg/ml (0-100 pg/ml), insulin 13.9 mcunit/ml(0-14mcunit/ml) fasting glucose 93mg/dl(70-110mg/dl), somatostatin 13 pg/ml (10-22 pg/ml), Chromogranin A 5 ng/ml(0-50ngml), PP 1755 pg/ml (0-435 pg/ml). A CT of the abdomen/pelvis was negative for duodenal or pancreatic masses however an endoscopic ultrasound detected 4 isoechoic pancreatic masses with the largest being 9mm x 5 mm. A FNA cytology reported a neuroendocrine tumor. At that time, the clinical decision was partial pancreatectomy. The pathology confirmed 4 macroadenomas with the largest diameter of 8 mm without positive lymph nodes. The microscopic evaluation revealed multiple foci of hyperplastic cells positive by immunostaining for glucagon but negative for others. She was scheduled to recheck PP levels and glucagon 3 months after surgery.

Discussion:
Our case presentation re-opens the discussion about the significance of elevated PP in MEN-1. PP was elevated 3 times the normal limit which elicited a radiologic workup and the identification of an occult PET. On the other hand, PP is a suboptimal marker to distinguish between functional and non functional PETs (4), and the diagnosis relies on immunopathology. Glucagonoma was an unexpected finding because of its low prevalence in MEN-1. However biopsies of pancreatic tissue in patients with MEN-1 have reported hyperplastic foci positive for glucagon (considered to be premalignant lesions) in up to 85% of asymptomatic cases (5).

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Nothing to Disclose: LPG-V, GL

Pub # P3-252

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Neuroendocrine Tumors (1:30 PM-3:30 PM)

Title Post-Surgical Follow-Up for the Diagnosis of Recurrence in Patients with Malignant Insulinoma

Author String A Buffet, D Vezzosi, J-C Maiza, S Grunenwald, A Bennet, N Carrere, P Caron
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Body Context: The post-surgical follow-up of malignant insulinomas is often marked by recurrence after initial remission. However, the criteria for the diagnosis of recurrence of these malignant endocrine tumors are not well defined in the literature, unlike diagnostic criteria for hyperinsulinemic hypoglycaemia (Cryer 2009). We retrospectively reviewed the criteria that allowed the diagnosis of recurrence in 4 patients with malignant insulinoma after initial post-operative remission. Patients and methods: At the initial diagnosis, 2 patients had a metastatic insulinoma (1 in the liver, 1 in lymph nodes) and 2 patients had a single tumor whose radiological presentation was benign. After Whipple's pancreatoduodenectomy (n=4), lymph node resection (n=3) and left hepatectomy (n=1) patients were considered in remission, defined as R0 resection and no clinical or biological post-operative hypoglycaemia. Results: The recurrence occurred after 14.8 ± 8.5 months (m \pm sd). Three patients had clinical signs of hypoglycaemia, but only one patient had a proved fasting hypoglycaemia (1.26 mmol/L). The remaining three patients did not reach the classical criteria of hyperinsulinemic hypoglycaemia during a 72 hour-fast test (n= 2) or serial plasma glucose, insulin and C-peptide measurements (every 4 hours for 24 hours) : the lowest plasma glucose levels in these three patients were 3.2, 2.5, and 4.3 mmol/L with concomitant C-peptide levels of 0.3, 0.5, and 0.8 ng/mL, respectively. For the 2 patients who had a 72 hour-fast test, serum beta-hydroxybutyrate concentration had reached the threshold of 2700 [μ mol/L. At the same time, radiological (CT scan n = 3, MRI scan n = 1) evaluation revealed lymph nodes (n= 2) or liver metastases (n= 2) attesting recurrence of malignant insulinoma. Conclusion: Based on the reported data, we suggest that after surgical remission the diagnosis of recurrence of malignant insulinomas cannot be based only on clinical and biological criteria but radiological evaluation by CT or MRI scan is mandatory during post surgical follow-up of patients.

Nothing to Disclose: AB, DV, J-CM, SG, AB, NC, PC

Pub # P3-253

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Neuroendocrine Tumors (1:30 PM-3:30 PM)

Title Clinical Characteristics of 86 Patients with Multiple Endocrine Neoplasia Type 1 (MEN-1) and Insulinomas

Author String D Vezzosi, C Cardot-Bauders, A Murat, M Bertholon-gregoire, A Tabarin, P Niccoli, N Levy-Bohbot, L Groussin, P Bouchard, P Chanson, P Lecomte, I Guilhem, E Mirallie, A Bennet, F Pattou, F Borson-Chazot, F Goudet, E Baudin, P Caron
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Body

Context:
Insulinomas are part of MEN-1 in 10% of the patients. The aim of our study was to describe the clinical features of insulinoma in MEN-1 patients.

Patients
86 patients, 57 female and 29 male aged 32±16 years (5-76), with MEN-1 and insulinoma from 30 French centers were retrospectively studied. All patients had MEN-1, symptomatic hypoglycemia and biochemical criteria of insulinoma according to standardized definitions.

Results
34 patients (40%) described exclusively neuroglycopenic symptoms, 8 patients (9%) reported only autonomic symptoms and 44 patients had both of these symptoms. Hypoglycemia occurred during a period of fast or physical exercise in 22 patients (25%), after meals only in 5 patients (6%) and was unrelated to meal-time in 59 patients (69%). Biochemical diagnosis was established at the time of spontaneous hypoglycemia in 50 patients and during a fast test in 36 patients. The fast test lasted 17±15 hours (2-53), and it had to be prolonged beyond 48h in only two patients. 73 insulinomas were classified as benign or uncertain behavior according to WHO criteria, 7 patients (8%) had metastatic insulinoma and 6 patients were not operated. Insulinoma was unique in 54 operated patients.
At the time of the diagnosis, biological hyperparathyroidism was present in 55 patients (64%), a pituitary adenoma in 27 patients (31%), an adrenal adenoma in 10 patients (12%), a gastric or thymic carcinoid tumor in 4 patients (5%). Other symptomatic functional pancreatic endocrine tumors were found in 19 patients (22%). Insulinoma was the first clinical manifestation of MEN-1 in 50 patients (58%).
23 patients (27%) had no extra-pancreatic features of other MEN-1-related disorders, and among them 14 patients (16%) aged 25 ± 18 years (5-58) presented only a single insulinoma and other manifestations of MEN1 developed a few year later.

Conclusion
MEN1-insulinoma is more frequent in females and can be revealed at any age with a higher prevalence in young adults. Isolated autonomic symptoms or postprandial hypoglycemia do not rule out the diagnosis. When spontaneous hypoglycemia does not occur, a 72-hour fast test must be performed, since hypoglycemia occurred in 2% of the patients after 48 hours of fast. Insulinoma is frequently the first clinical manifestation of MEN-1. Asymptomatic hyperparathyroidism is often associated with insulinoma. However 16% of the patients presented a single pancreatic endocrine tumor without any other manifestation of MEN-1.

Nothing to Disclose: DV, CC-B, AM, MB-G, AT, PN, NL-B, LG, PB, PC, PL, IG, EM, AB, FP, FB-C, PG, EB, PC

Pub # P3-254

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Neuroendocrine Tumors (1:30 PM-3:30 PM)

Title New Kindred MEN1 Presenting as Metastatic Pancreatic Cancer and Postoperative Hypophosphatemia

Author String CR Palmeiro, M Schwarcz
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Body

Background:
Multiple Endocrine Neoplasia Type-I (MEN1) is a rare yet important cause of hyperparathyroidism; representing approximately two-to-four percent of cases (1). The classic laboratory abnormalities seen in hyperparathyroidism are hypercalcemia, hypophosphatemia, and an elevated intact PTH level. However, in large abdominal surgeries, superimposed electrolyte abnormalities can be precipitated by aggressive volume expansion, transfusions, acute-critical illness, and inflammatory cytokines (2,3).

Clinical Case:
A 63-year old male without a significant past medical history and a family history of paternal nephrolithiasis presented with epigastric pain. A computed tomography (CT) scan of the abdomen revealed a 5.6 centimeter mass in the pancreatic body. Both histologic samples from FNA of the pancreatic mass and local lymph node, as well as somatostatin-receptor scintigraphy, strongly suggested a diagnosis of neuroendocrine tumor with metastases to regional lymph nodes and the liver. The patient then underwent an open Whipple's resection with a splenectomy, an omentectomy, and a liver biopsy. Microscopic examination demonstrated a well-differentiated endocrine tumor. Immunohistochemical studies were positive for chromogranin, synaptophysin and trace for somatostatin, while negative for insulin, gastrin, and glucagon.

Post-operatively the patient developed persistent hypophosphatemia (nadir 0.8 mg/dl, 2.3-4.7 mg/dl), in the face of normocalcemia (ionized calcium 4.7 to 5.2 mg/dL, 4.5-5.3 mg/dL) and normomagnesemia (1.8 to 2.4 mg/dL, 1.6-2.6 mg/dL). A twenty-four hour urine collection for phosphorous was high (2.3 g/24 hours, 0.4-1.1 g/24 hours) and the intact PTH was found to be markedly elevated (1523.5 pg/mL, 7-53 pg/mL). Subsequently, the patient underwent total parathyroidectomy with autograft implantation into his nondominant forearm. Histological specimens revealed three hyperplastic parathyroid glands with fragments of the fourth.

Conclusion:
This is a case of MEN1 presenting as metastatic, nonfunctioning entero-pancreatic carcinoma and post-operative hypophosphatemia. Hypercalcemia was likely masked by acute-critical illness after major abdominal surgery. Clinical lessons include:

1. Review a case, with a unique presentation, that should raise suspicion for MEN1.
2. Understand how post-operative electrolyte abnormalities, like hypocalcemia, can alter the classic laboratory findings of hyperparathyroidism.

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Nothing to Disclose: CRP, MS

Pub # P3-255

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Neuroendocrine Tumors (1:30 PM-3:30 PM)

Title Endoscopic Ultrasound-Guided Fine Needle Tissue Acquisition (EUS-FNTA) Using a 19-G Needle for Histological Grading of Pancreatic Endocrine Tumors (PETs): A Prospective Study

Author String A Larghi, A Bianchi, A Fusco, F Lugli, D Iacovazzo, V Cimino, A D'Uonno, G Capurso, A Carnuccio, G Delle Fave, G Costamagna, G Rindi, A Pontecorvi, L De Marinis
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Body Endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) is increasingly used in the diagnosis of pancreatic endocrine tumors (PETs). Ki-67 expression, an important prognostic factor according to the WHO classification, is difficult to be determined on cytological specimens. We prospectively evaluated the feasibility and yield of EUS-guided fine needle tissue acquisition (EUS-FNTA) using a 19-gauge needle to obtain tissue samples for histological diagnosis and Ki-67 expression in patients with suspected PETs. Twenty-five consecutive patients (mean age 54±15 yrs; M/F 11/14) with suspicious of PETs on previously performed imaging studies, underwent, between August 2009 and September 2010, EUS-FNTA using a 19G needle (Echotip Ultra, CookEndoscopy, Winston-Salem,NC). The collected specimens were placed directly in formalin for histological examination. All patients had a single lesion localized in the pancreatic head in 5, in the uncinate process in 2, in the isthmus in 4, in the body in 5, in the tail in 6, and at the body-tail junction in 3. In 18 patients EUS-FNTA was performed using the conventional linear echoendoscope (GF-UCT140-AL5, Olympus Medical System Corp., Tokyo, Japan), while in the other 7 patients the newly developed forward viewing echoendoscope (XF-UCT-160J-AL5, Olympus) was used. The mean size of the biopsied lesions was 15.6±7.3 mm (range 7-100 mm). Overall, EUS-FNTA could be performed in all patients without complications. A mean of 2.7 ±0.5 passes per patient was performed. Tissue samples for histological examination were retrieved in 23 out of the 25 patients (92%) and were sufficient to make a diagnosis of NETs in all these patients. Ki-67 determination could be performed in 21 of the 23 patients (91.3%) with available tissue from EUS-FNTA. In the 6 patients who underwent surgery, pre- and post-surgical Ki-67 values were concordant in 4, while in 2 patients upgrading of the Ki-67 in the post-surgical specimen occurred. In patients with suspected PETs, retrieval of tissue specimens with EUS-FNTA using a 19G needle is safe and feasible, with a high diagnostic accuracy even in those with small lesions localized in difficult segment of the pancreas to be sampled. Determination of Ki-67 on tissue samples acquired through this technique can be helpful in guiding further management decision.

Nothing to Disclose: AL, AB, AF, FL, DI, VC, AD, GC, AC, GDF, GC, GR, AP, LDM

Pub # P3-256

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Neuroendocrine Tumors (1:30 PM-3:30 PM)

Title Distinct Cut-Off Values of Ki-67-Index for NET-Grading Predict Long-Term Outcome in Gastrointestinal Neuroendocrine (Carcinoid) Neoplasms

Author String U-F Pape, A Rinke, S Maasberg, N Begum, ME Pavel, C Auernhammer, M Anlauf, M Schott, S Ezzidin, B Wiedenmann, H Lehnert
Charité, University Medicine, Berlin, Germany; Philipps University, Marburg, Germany; University Clinic Schleswig-Holstein, Campus Lübeck, Lübeck, Germany; Ludwig-Maximilians-University, München, Germany; Heinrich-Heine-University, Düsseldorf, Germany; University Clinic, Bonn, Germany

Body
Introduction: Histopathological grading (G) of neuroendocrine neoplasms (NEN, formerly termed carcinoid tumors) according to the novel WHO classification of 2010 as well as the recent AJCC/UICC and ENETS classifications is based on immunohistochemical staining of the cell cycle dependent marker Ki-67 antigen or the mitotic index. Close margins between G1- and G2-NEN, however, often pose difficulties in prognostic stratification.
Aim: We analyzed the Ki-67 values in a large multicentric cohort from Germany using different cut-off values and correlated the numbers with clinical outcome data i.e. survival since initial diagnosis.
Methods: 2009 patients with histologically proven NEN diagnosed since 1999 from 28 centres in Germany registered in the German NET registry were retrospectively analyzed for Ki-67 values, histopathological classification, clinical data and information on overall and NET-specific using Kaplan-Meier analysis and log rank testing.
Results: Ki-67-grading showed significantly poorer survival for G3-NET (Ki67>20%) as compared to G1- (<3%) and G2-NET (3-20%; p<0.001), while G1- and G2-NET did not differ significantly (p=0.112) using the published cut-off values. However, raising the cut off between G1 and G2 to 5% resulted in significant differentiation of G1 from G2-NET (p<0.001). This was particularly shown for NEN originating in the pancreas.
Conclusion: Grading of NEN according to recent recommendations with cut off margins of 2% for G1-NET often fails to withstand prognostic significance in statistical analysis even in this large cohort. Raising the cut off to 5% for G1-NEN significantly improves differentiation of overall survival in G1- and G2-NEN. This approach proves to be of prognostic significance and could improve the predictive value of NEN grading and therapeutic stratification particularly since novel molecular therapies for NEN are on the rise.

Sources of Research Support: The German NET-Registry receives funding from Novartis Pharma GmbH, Germany, and Ipsen Pharma GmbH, Germany.

Nothing to Disclose: U-FP, AR, SM, NB, MEP, CA, MA, MS, SE, BW, HL

Pub # P3-257

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Neuroendocrine Tumors (1:30 PM-3:30 PM)

Title A Randomized Cross-Over Study in Patients with Neuroendocrine Tumors (NETs) To Assess Patient Preference for Lanreotide Autogel Given by Self/Partner or Given by Healthcare Professional

Author String V Johanson, B Wilson, A Abrahamsson, C Jianu, J Calissendorff, N Wall, H Gronbaek, J Florholmen, D Granberg, V Aberg, A Ohberg
Sahlgrenska University Hospital, Gothenburg, Sweden; Odense University Hospital, Odense, Denmark; Karolinska Institutet, Karolinska University Hospital, Huddinge, Sweden; St Olav Hospital, Trondheim, Norway; Karolinska Institutet, Karolinska University Hospital, Solna, Sweden; University Hospital, Linköping, Sweden; Aarhus University Hospital, Aarhus, Denmark; University Hospital of North Norway, Tromsø, Norway; University Hospital, Uppsala, Sweden; Ipsen AB, Stockholm, Sweden

Body

Introduction/Background
Ready-to-use Lanreotide Autogel provides a possibility of self/partner administration for patients with NETs. This is anticipated to reduce treatment cost and negative impact on daily life.

Aims
To assess the proportion of patients preferring self/partner injections and to describe the impact on costs, efficacy and safety.

Materials and Methods
26 patients with symptomatic NETs treated with a stable dose of Lanreotide Autogel, 90 or 120 mg, every 4 weeks were randomised to start with either self/partner or healthcare administration. After 3 injections patient switched administration method. The total study duration for each patient was 7.5-8.5 months including 2-3 training injections.

Results
According to patient diaries, 22 of 25 (88%; 95% CI 68.8-97.5) evaluable patients (12 F, 13 M; mean age 61.6 (38-77)) preferred self/partner injections mainly due to increased independence. Cost savings were primarily caused by a reduced number of visits to the clinic. No difference in efficacy and safety were noted between administration methods.

Conclusion
Patients with NETs were able to self/partner administer Lanreotide Autogel without any impact on efficacy or safety. 88% of the patients preferred self/partner injections on a regular basis. This administration method provides a good alternative for suitable patients in order to increase patient independence and reduce visits to the clinic.

Sources of Research Support: Ipsen AB, Stockholm, Sweden.

Disclosures: VJ: Study Investigator, Ipsen. BW: Study Investigator, Ipsen. AA: Study Investigator, Ipsen. CJ: Study Investigator, Ipsen. JC: Study Investigator, Ipsen. NW: Study Investigator, Ipsen. HG: Study Investigator, Ipsen. JF: Study Investigator, Ipsen. DG: Study Investigator, Ipsen. VA: Employee, Ipsen. AO: Employee, Ipsen.

Pub # P3-258

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Neuroendocrine Tumors (1:30 PM-3:30 PM)

Title Population Pharmacokinetics of Lanreotide Autogel in Patients with Carcinoid Neuroendocrine Tumors

Author String I Troconiz, MJ Garrido, J Ramis, JM Cendros, R Obach
Ipsen, Sant Feliu de Llobregat, Spain; University of Navarra, Pamplona, Spain

Body

Background and purpose. Lanreotide Autogel^(R) (LA) at doses of 60, 90, and 120 mg injected by deep subcutaneous administration is currently being used for the treatment of acromegaly and reduces the symptoms associated with carcinoid syndrome. The aim of this study is to explore the population pharmacokinetic (popPK) characteristics of LA in patients with carcinoid neuroendocrine tumors (CNETs) as a first step to understand the response vs exposure relationships.

Patients and Methods. The study population consist on 71 patients with CNETs that received six subcutaneous injections of LA in intervals of 28 days. During the first two administrations LA was injected at the dose of 90 mg. Afterwards dose levels of 60, 90, or 120 mg were administered according to symptom response. Serum concentrations of lanreotide were obtained at the end of each dosing interval (every 28 days) A total 353 serum lanreotide concentrations were used during the popPK analysis.

The popPK model previously established for LA in healthy volunteers(1) was used to describe the pharmacokinetic (PK) characteristics of LA in patients with CNETs. Due to the sparse nature of the current data, the PK parameters estimated in healthy volunteers were fixed, with the exception of the absorption PK parameters, which was allowed to differ from the previous estimate obtained in volunteers. All the analyses were performed with the software NONMEM version VII.

Results. The model and PK parameter estimates obtained in healthy volunteers provided an adequate description of the individual PK profiles of lanreotide in patients with CNETs. However, the observed concentrations of lanreotide in patients with CNETs tended to be higher than those predicted by the typical population profiles without inclusion of covariates, suggesting that bioavailability could be somewhat increased in patients with CNETs compared to volunteers.

Conclusions. These results indicate that the pharmacokinetic characteristics of lanreotide after LA injection are at least similar between healthy volunteers and patients with CNETs, although a somewhat higher drug exposure is observed in these patients.

(1) Troconiz et al., Clinical Pharmacokinetics 2009; 51:62.

Disclosures: JR: Employee, Ipsen. JMC: Employee, Ipsen. RO: Employee, Ipsen. Nothing to Disclose: IT, MJG

Pub # P3-259

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Neuroendocrine Tumors (1:30 PM-3:30 PM)

Title Multiple Primary Endocrine Tumors in a Single Individual -- A Novel MEN Syndrome?

Author String CA Hurd, AM Innes, JL Paseika, CJ Symonds
University of Calgary, Calgary, Canada; University of Calgary, Calgary, Canada; University of Calgary, Calgary, Canada

Body
Background: Multiple endocrine tumor syndromes are associated with several known genes. The yield of genetic testing varies depending on the clinical presentation, but is high in patients presenting with classic von Hippel Lindau (VHL) syndrome.
Clinical Case: During the workup of an elevated PSA, a 66-year-old man was incidentally found to have a 3.8 cm left kidney mass suspicious for a renal cell carcinoma (RCC) and a 1.5 cm mass in the anterior body of the pancreas suspicious for a neuroendocrine tumor. Nephrectomy was complicated by an intraoperative hypertensive crisis (peak BP 270/140), despite no previous history of hypertension. Pathology confirmed a clear cell renal carcinoma (grade 2/4, pT1a). A second look at the preoperative CT scan showed a 2 cm right adrenal mass that was not originally reported. Elevated plasma free and urine metanephrines were elevated, consistent with a pheochromocytoma. Further investigations revealed an elevated calcium and PTH, a normal gastrin, and a fasting glucose of 2.5 mmol (reference 3.6-6.1 mmol/L). Interestingly, he did not have classic symptoms of fasting hypoglycemia. After alpha blockade, an open right adrenalectomy and distal splenic-preserving pancreatectomy were performed. Final pathology confirmed a benign 2 cm right-sided pheochromocytoma and a 1.8 cm pancreatic well-differentiated neuroendocrine tumor (stained for insulin and gastrin). The patient remains hypercalcemic, and is currently awaiting parathyroidectomy. Past medical history was significant for a deep vein thrombosis attributed to idiopathic polycythemia. There was no family history of RCC or endocrine neoplasms.
The best unifying clinical diagnosis was VHL. However, genetic testing did not reveal a mutation or deletion in the VHL gene. Additionally, a VHL mutation was not found in the pancreatic tumor, thus essentially excluding mosaicism. As primary hyperparathyroidism is associated with other endocrine tumor syndromes, we proceeded with further genetic workup. However, mutations in other genes commonly associated with multiple endocrine tumors - including SDHB, SDHC, SDHD, MENIN, RET, and Cdkn1b- were not found.
Conclusion: Four primary tumors in a single individual strongly suggests an underlying genetic predisposition. Mutations in other components of the HIF pathway are plausible candidates for future study. We will discuss our multidisciplinary approach to this patient with atypical tumor associations.

Nothing to Disclose: CAH, AMI, JLP, CJS

Pub # P3-260

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Neuroendocrine Tumors (1:30 PM-3:30 PM)

Title Tall-Cell Variant Papillary Thyroid Carcinoma Associated with MEN1

Author String TD Hoang, VQ Mai, PW Clyde, KM Shakir
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Body **Background:** Multiple endocrine neoplasia type 1 (MEN1) is characterized by a predisposition to tumors of the parathyroid glands, anterior pituitary, and pancreatic islet cells. We report a case of tall-cell variant papillary thyroid carcinoma (PTC) in a patient with MEN1.

Clinical case:
A 21 y/o female with a history of hyperparathyroidism, and nephrolithiasis was noted to have a palpable thyroid nodule. She had no complaints of galactorrhea, headaches, visual field changes, or peptic ulcer disease. Family history was significant for two family members with MEN1: father with hyperparathyroidism, a pancreatic mass, and pituitary microprolactinoma; and paternal grandfather with parathyroid adenoma and a pancreatic mass. On examination, her vital signs were normal. Neck examination revealed a right thyroid nodule without palpable lymph nodes. Thyroid ultrasound confirmed a 1.4 cm x 1.3 cm x 1.2 cm thyroid nodule in the right lobe and four enlarged parathyroid glands. There were lymph nodes with benign features bilaterally. Abdominal CT scan showed 3 lesions in the body of the pancreas. MRI of the pituitary showed a 3 mm microadenoma that is non-functioning on evaluation. FNA of the thyroid nodule confirmed papillary thyroid carcinoma. Serum calcium was 10.5 mg/dL (nl 8.4-10.2) and parathyroid hormone was 183 pg/mL (n 14-72).

The patient underwent total thyroidectomy with central neck dissection, cervical thymectomy, total parathyroidectomy with parathyroid autotransplantation to the left forearm. Pathology revealed multifocal tall cell variant papillary carcinoma (1cm on the right, 0.1 cm on the left) with multiple positive lymph nodes. She was treated with I¹³¹ 164 mCi. BRAF V600E mutation result is pending.

Endocrine pancreatic evaluation showed normal chromogranin, glucagon, gastrin, and other GI hormones. Endoscopic ultrasound-guided biopsy of the pancreas mass revealed neuroendocrine lesions. Patient then underwent distal pancreatectomy. Histopathology confirmed neuroendocrine tumors. Genetic analysis confirmed a heterozygous sequence associated with MEN1 in a dominant mode of inheritance.

Conclusions: Three previous case reports did not find an etiological association of PTC with MEN1; however, it was suggested that loss of the MEN1 tumor suppressor gene was not required for the oncogenesis of PTC. This is the first case report of tall-cell PTC associated with MEN1. This case may suggest that patients with MEN1 should be screened for thyroid neoplasms.

Kim HJ et al. Yonsei Med J Vol 49. No 3, 2008.

Nothing to Disclose: TDH, VQM, PWC, KMMS

Pub # P3-261

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Neuroendocrine Tumors (1:30 PM-3:30 PM)

Title Multiple Endocrine Neoplasia Type 1 Associated with Atypical Lipoma

Author String TD Goncalves, DM Lourenco, Jr, RA Toledo, R Falzoni, SPA Toledo
University of São Paulo School of Medicine, São Paulo, Brazil; University of São Paulo School of Medicine, São Paulo, Brazil

Body **Introduction:** Multiple Endocrine Neoplasia type 1 (MEN1) is a disease characterized classically by the development of tumors in three endocrine glands: parathyroid, hypophysis and pancreas/duodenum. It can be of familial occurrence or sporadic. The familial MEN-1 is an autosomal dominant disorder which has the MEN1 gene as its main responsible. Several other endocrine and no-endocrine tumors were related to NEM1 as carcinoid tumors, dermic tumors (lipomas, angiofibromas, collagenomas) of the CNS and of the mesenchymal tissue. **Case:** VAIN - 32 years old/female, at 26 diagnosed with galactorrhea and at 29 prolactinoma (PRL: 379,9 ng/dL; VR: up to 15; microadenoma). At 29 complained of a painful tumorigenicity in the left inferior member. A RNM revealed an expansive development in the womb proximal to the lateral vast muscle (13x5,7x4,3cm), suggesting liposarcoma. Another tumor developing was found in the right subscapular/interscapular-vertebral region (7,3x6,2x1,9cm), suggestive of lipoma. An exeresis of the tumor in the thigh was performed and the anatomo-pathological was conclusive for atypical lipomatosis tumor (liposarcoma well differentiated). In post-operative exams asymptomatic primary hyperparathyroidism was diagnosed (total Calcium: 10,6 mg/dL; VR: 8,5-10,2; PTH: 153; VR: 13 - 62 pg/ml; scintigraphy: hypercaptation of right inferior parathyroid) being submitted to subtotal parathyroidism. Follow-up was started when patient was 31 years old. Echo-Endoscopy revealed a non-functioning neuroendocrine pancreatic tumor. After 2 years, there was not local recurrence of the atypical lipoma. **Discussion:** Lipomas occur in 20-30% of the patients with NEM1. They are usually multiple, benign and they are located in the subcutaneous tissue or, more rarely, visceral. When surgically removed it usually has no recurrence. Cutaneous tumors can help in the diagnosis of NEM1 when they appear prematurely and before clinical manifestations of tumoral hormone secretion. Atypical lipomas include 45% of all of the liposarcomas. In spite of the absence of distant metastases these tumors can be locally aggressive. This case suggests that periodic surveillance on dermic tumorigenicity in patients with NEM1 can aid in the diagnosis and precocious treatment of atypical lipomas.

Nothing to Disclose: TDG, DML, RAT, RF, SPAT

Pub # P3-262

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Neuroendocrine Tumors (1:30 PM-3:30 PM)

Title The Beneficial Effects of Long-Acting Octreotide in a Patient with Concomitant Metastatic Neuroendocrine Tumor and Anemia Due to Lower Digestive Tract Bleeding

Author String RK Crowley, S Gupta, L McGowan, D O'Shea, G Tamagno
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Body Somatostatin analogues (SA) represent the most effective medical treatment for the control of neuroendocrine tumor (NET) symptoms, like carcinoid syndrome. Recently there has been evidence of lengthened time to tumor progression in patients with midgut carcinoid treated with octreotide. Nevertheless, a number of new therapeutic indications and the clinical effectiveness of SA for other clinical conditions are appearing. A 69-year-old man was investigated for anaemia (haemoglobin: 8.0 g/dl). He required blood transfusion every 2-3 weeks. He had a medical history of peripheral vascular disease, coronary artery disease and by-pass graft, decreased left ventricular function, and pulmonary hypertension. Following a set of investigations, chronic digestive tract bleeding likely secondary to angiodysplasia was diagnosed but surgical approach was not feasible. The radiological investigations incidentally discovered abdominal and inguinal lymph node metastases and subsequent lymph node biopsy revealed metastatic NET. Unfortunately, the primary tumor remained unidentified. Treatment with long-acting octreotide was established. The need for blood transfusion: dramatically decreased starting from the first month of treatment with long-acting octreotide. His haemoglobin levels improved and remained stable around 10.0 g/dl over the following 12 months. Since commencement of octreotide, he required one blood transfusion every 4-5 months only. No adverse events occurred and the patient remained stable from a NET-related point of view. To the best of our knowledge, this is the first report of a serendipitous effect on anaemia due to chronic lower digestive tract bleeding achieved by long-acting octreotide treatment prescribed for metastatic NET. This report is consistent with the literature reporting a potential indication of SA for the treatment of lower digestive tract bleeding from a known or unknown source. In our opinion, this report shows that treatment with SA in patients with lower digestive tract bleeding can be beneficial if the surgical approach is not feasible and only palliative therapies are available.

Nothing to Disclose: RKC, SG, LM, DO, GT

Pub # P3-263

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Neuroendocrine Tumors (1:30 PM-3:30 PM)

Title Recurrent Acute-Onset Cushing Syndrome 6 Years after Removal of a Thymic Neuroendocrine Carcinoma: From Ectopic ACTH to CRH Secretion

Author String C Schalin-Jantti, JT Arola, T Sane
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Body
Background: Concomitant ACTH- and CRH-secretion in ectopic Cushing's syndrome is extremely rare. We describe a case where acute Cushing's syndrome caused by a 3.6 cm ACTH-producing thymic neuroendocrine carcinoma turned into acute Cushing's syndrome compatible with CRH overproduction after 6 years of remission.
Clinical case: A 41-yr old male developed acute onset Cushing's syndrome within a few days. Clinical presentation and initial tests (hypokalemia, increased 24hr urinary cortisol secretion (31800 nmol/l), serum cortisol (1938 nmol/l) and plasma ACTH (4425 ng/l) were consistent with ectopic ACTH-syndrome. CT scan showed a 3.6 x 1.9 cm thymic tumor which was successfully operated. ACTH (48 g/l) was normal on the first postoperative day. Histopathological examination was compatible with a well-differentiated neuroendocrine carcinoma, MIB-1 proliferation index was 5-10 % and ACTH-staining strongly positive. The patient was regularly followed up and in remission for 6 years. He then again developed acute onset Cushing's syndrome with increased 24h urinary cortisol (15602 nmol/l) and serum cortisol (1745 nmol/l). DOPA-PET showed a local uptake in the previously operated mediastinal region. The patient was reoperated. Unexpectedly, plasma ACTH remained increased (613 ng/l) on the first postoperative day and 24h urinary cortisol remained increased (36720 nmol/l). Histopathology showed recurrent neuroendocrine carcinoma, proliferation index was in the previous range (5-10 %). However, ACTH-staining was only weakly positive. Metopirone treatment was started to control hypercortisolism. Thereafter, symptoms and cortisol metabolism normalized within 1 week and metopirone was withdrawn. He is in remission as examined in January 2011, one year after the second operation and external radiotherapy.
Clinical Lessons: After the initial operation, ACTH concentration immediately dropped to normal. This is compatible with ectopic ACTH-secretion. The patient had a recurrence 6 years later, but this time ACTH remained increased after the operation suggesting incomplete tumor removal. However, ACTH secretion and 24h urinary cortisol normalized within two weeks. The clinical course was compatible with ectopic CRH-secretion and stimulation of pituitary ACTH secretion which gradually resolved.

Nothing to Disclose: CS-J, JTA, TS

Pub # P3-264

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Neuroendocrine Tumors (1:30 PM-3:30 PM)

Title Necrolytic Migratory Erythema as a Manifestation of Glucagonoma in a Patient with Multiple Endocrine Neoplasia Type 1: Report of a Case

Author String JIS Mota, P de Queiroz, M Lima, I Porto, TB Ferraz, JG Oliveira, DB de Menezes, AR de Sousa
Fortaleza General Hospital, Fortaleza, Brazil; Dona Libania Dermatology Center, Fortaleza, Brazil; Fortaleza General Hospital, Fortaleza, Brazil

Body
Background: MEN-1 is a rare hereditary disease characterized mainly by hyperplasia and/or multiple tumors of the parathyroid, endocrine pancreas, foregut-derived neuroendocrine tissues and anterior pituitary. The most common functioning pancreatic tumors observed in patients with MEN 1 are gastrinomas (50-70%) and insulinomas (20-40%). Glucagonomas, vasoactive intestinal peptide adenomas (VIPoma) and somatostatinomas appear to be rare (<5%).
Clinical case: A 32 years old woman was admitted to Endocrinology Department because of secondary amenorrhea for 8 years and family history of MEN-1 (a sister and a cousin). She had no positive past medical history and the physical examination was normal. The diagnosis of prolactinoma was made on the basis of high plasma prolactin levels (> 200 ng/ml, n4,79-23,3 ng/ml) associated with a macroadenoma visualized on MRI. Therapy with cabergoline was initiated. Laboratory analysis at this period showed serum calcium of 11,5 mg/dl (n8,5-10,5 mg/dl), raised PTH (279 pg/mL, n10-69 pg/ml) and raised Alkaline phosphatase (462 U/L, n100-290 U/L). A 99mTc-sestamibi scintigraphy showed a focal area of increased uptake in the right inferior parathyroid gland, confirming the diagnosis of primary hyperparathyroidism. Patient was submitted to parathyroidectomy. In the same period, laboratory analysis showed normal basal gastrin levels (36,5; 67,2 pg/mL, n13-115 pg/mL) and impaired fasting glucose (115 mg/dl, n < 100 mg/dl).
After a four-year period, the diagnosis of diabetes was confirmed (FPG 129; 133 mg/dl, n < 126 mg/dl) and the patient developed an abdominal cutaneous eruption with erythematous plaques. Most of these lesions healed, leaving residual hyperpigmentation. The clinical-histopathologic evaluation in the dermatology department was compatible with the diagnosis of necrolytic migratory erythema and fasting plasma glucagon level measured by radioimmunoassay was 95,3 pg/ml (n < 60 pg/ml). Abdominal CT and MRI were normal. OctreoScan demonstrated two areas with high uptake in the upper abdomen, leading to the diagnosis of glucagonoma. The patient is waiting for an endoscopic ultrasonography for preoperative tumor localization.
Conclusion: We report a female with a variant type of MEN-1 composed initially of prolactinoma and primary hyperparathyroidism. The diagnosis of necrolytic migratory erythema is important, since it might be the clue for early detection of glucagonoma or of extra-pancreatic glucagon-secreting tumors.

Nothing to Disclose: JISM, PdQ, ML, IP, TBF, JGO, DBdM, ARdS

Pub # P3-265

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Neuroendocrine Tumors (1:30 PM-3:30 PM)

Title Acquired ACTH-Dependent Cushing Syndrome in Metastasized Carcinoid Tumor

Author String P Paul, S Van Uum, M Sanatani, CA McLean
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Body

Background: In ACTH-dependent Cushing's syndrome, the elevated ACTH production originates from the pituitary or from an ectopic source. Rarely, ectopic ACTH production may originate from carcinoid tumors. Clinical case: In June 2009, a 56 year old man was diagnosed with a pancreatic neuroendocrine carcinoma (Ki-67 index 24%), metastasized to liver and bones, and clinical carcinoid syndrome at diagnosis. He underwent 4 chemotherapy cycles, followed by radioactive octreotide (ending June 2010) and was maintained on long-acting somatostatin analogue.

In June 2010, his family doctor diagnosed diabetes mellitus, and new onset hypertension and hypokalemia was noted. He remained unwell and severe hypokalemia (1.7 mmol/L) twice necessitated intravenous potassium administration in his local Emergency Room. An endocrinology consult, requested for hyperglycaemia, raised suspicion for Cushing's syndrome. Baseline serum cortisol was 3411 (normal 119-618) nmol/L, with minimal suppression to 2742 nmol/L on the 1 mg dexamethasone suppression test (DST). Urinary cortisol excretion was elevated at 77,285 (normal 106-346) nmol/day. The elevated ACTH (70.62 pmol/L) confirmed ACTH dependent Cushing's syndrome. Cortisol did not suppress following an 8 mg DST. Pituitary MRI, CT scan of abdomen, pelvis, and thorax, and an octreotide scan showed only the unchanged metastasized neuroendocrine tumor.

The clinical presentation, laboratory investigations and normal pituitary imaging suggested new onset ACTH-dependent Cushing's syndrome, most likely from new onset ACTH production from the neuroendocrine tumour. Review of the original pathology from 2009, before onset of hyperglycaemia and hypokalemia, did not show ACTH positivity. Because of the poor prognosis, adrenalectomy was not considered, ketoconazole was initiated resulting in a mild cortisol decrease. One month later, the patient was admitted with hypotension and hypokalemia, and died shortly after.

Discussion: This case suggests that a neuroendocrine carcinoma with associated carcinoid syndrome can transform into an ACTH producing tumor during monitoring/treatment. We speculate that the octreotide treatment may have induced differentiation of the neuroendocrine malignancy.

Conclusion: In case of hyperglycemia and hypokalemia, secondary causes must be suspected. With the history of carcinoid tumor, ectopic ACTH production must be considered, even if Cushing's syndrome was not present when the tumor was originally diagnosed.

Nothing to Disclose: PP, SVU, MS, CAM

Pub # P3-266

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Neuroendocrine Tumors (1:30 PM-3:30 PM)

Title Ectopic and Cyclic Cushing Syndrome Caused by a Thymic Carcinoid Tumor

Author String GH Telo, AP Cioffi, TC Rodrigues, MA Czepielewski
Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil; Faculdade de Medicina, UFRGS, Porto Alegre, Brazil

Body Ectopic ACTH Syndrome (EAS) is responsible for 10-20% of Cushing's syndrome (CS) and some patients have occult tumors. The aim of this study was to report a patient with CS initially occult which the diagnosis was established after 3 years. A 23-yr-old woman was referred with a 1-yr history of weakness, facial swelling, abdominal purple striae and weight gain. Plasma cortisol at overnight test, midnight test and urinary cortisol were 45 [micro]g/dl, 97 [micro]g/dl and 15.627 [micro]g respectively. In the next 4 months, she had three periods of worsening and remission, setting the framework of cyclic CS. Plasma ACTH was 90 pg/ml and at this moment the magnetic resonance imaging (MRI) of pituitary gland was normal. Peripheral test of DDAVP identified an increase of ACTH (>100%), but without central gradient in the inferior petrosal sinus catheterization. EAS was suspected and the search for the primary site was on track. Computed tomography (CT) of the abdomen, thorax and mediastinum, thyroid ultrasound, fiber optic bronchoscopy, endoscopy, colonoscopy and bone, MIBG and octreoscan found no tumor as well calcitonin, 5-hydroxyindolacetic were normal. All images were repeated over 6 months and were normal. Due to the severity of the condition, she underwent bilateral adrenalectomy, with significant clinical improvement. After one year, during pregnancy, there was a significant elevation of ACTH levels (3930 pg/ml). Thorax CT after delivery showed a lesion in the anterior mediastinum (8x4cm). Biopsy with immunohistochemistry was positive for chromogranin A (at baseline, 300 ng/ml). Surgical resection showed a thymic carcinoid tumor. During the subsequent year, the patient did not have clinical evidence of metastasis and the chromogranin A levels were normal (5 ng/ml). The clinical spectrum of EAS is broad. Tumor has not been identified in 19% of the patients. The criteria for cyclic CS include three peaks and two troughs of cortisol and the term occult has been used to refer to tumors that were not initially apparent. An intrathoracic tumor is detected in 41-60%. Sometimes CT or MRI showed these tumors only after long term follow-up (6 m up to 12 yr). Mediastinal neuroendocrine tumor tends to be initially occult. Chromogranin A is a useful tumor marker since the diagnosis is made at follow-up and it must be considered in the diagnosis of occult CS.

Nothing to Disclose: GHT, APC, TCR, MAC

Pub # P3-267

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Neuroendocrine Tumors (1:30 PM-3:30 PM)

Title Successful Treatment of Severe Subacute Hypercortisolism Due to ACTH-Secreting Neuroendocrine Carcinoma Using a Staged Medical and Surgical Strategy

Author String JM Silva, A Grover, EK Alexander
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Body

Introduction: ACTH-secreting neuroendocrine tumors (NET's) result in hypercortisolism, which is often severe and life-threatening. Critical to a successful outcome, a combined medical-surgical strategy must be employed. The optimal timing of each treatment step, however, remains ill-defined. We describe a patient with severe hypercortisolism effectively treated via both medical and surgical approaches, who has since experienced long-term survival.

Clinical Case: A 65-year-old man was diagnosed with moderately-differentiated pancreatic neuroendocrine carcinoma metastatic to the liver. He underwent biliary stent placement and initiated experimental chemotherapy with temozolomide and everolimus. Over the next 7 months he developed hypertension, hyperglycemia, profound potassium wasting (>300meq/day), and severe proximal weakness. Serum cortisol was elevated and not suppressible with high-dose dexamethasone. ACTH concentration was elevated. A 24-hour urinary cortisol measurement was >9,000mcg/24hrs (3.5-45). Treatment with aldactone, insulin, prophylactic antibiotics, and potassium supplementation was initiated. Unfortunately, the tumor was deemed unresectable and bilateral adrenalectomy considered too high-risk in this critically ill individual. Therefore, medical control of the Cushing's syndrome was initiated. Ketoconazole (400 TID) and metyrapone (500 TID) were started, resulting in reduction of random serum cortisol to 27mcg/dL (normal <20mg/dL). Over 6 weeks the patient showed tremendous improvement and was able to reinstate chemotherapy. Unfortunately, a restaging CT four months later revealed worsening hepatic metastatic disease. Given the improvement induced by medical management of his Cushing's syndrome, the patient was recommended for bilateral adrenalectomy through the retroperitoneal approach. This was successful. His metabolic derangements resolved and he is maintained on glucocorticoid and mineralocorticoid replacement. The patient has been successfully managed for nearly 1 year since time of diagnosis and is currently being considered for an alternative chemotherapy regimen.

Clinical Lessons: Severe hypercortisolism is frequently described in cases of metastatic neuroendocrine malignancy. Most often, such cases exhibit extremely high mortality in the first 60 days. As our case illustrates, a staged approach utilizing medical and surgical therapies can successfully lead to long-term survival and improved quality of life for such patients.

Nothing to Disclose: JMS, AG, EKA

Pub # P3-268

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Neuroendocrine Tumors (1:30 PM-3:30 PM)

Title Multiple Endocrine Neoplasia Type 1 with Neuroendocrine Tumor of the Ovary

Author String JM Sharretts, WF Simonds, SJ Marx
National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD

Body

Background: Multiple endocrine neoplasia, type 1 (MEN-1) is an autosomal dominant condition associated with pituitary adenomas, primary hyperparathyroidism and enteropancreatic neuroendocrine tumors. Carcinoid tumors of the bronchus and thymus are also known to occur. In patients with an identifiable germline mutation of the MEN1 gene located at 11q13, these tumors demonstrate loss of heterozygosity (LOH) at the gene locus. Neuroendocrine tumor of the ovary has not previously been described in association with this syndrome.

Clinical case: 24 year-old female was recently diagnosed as an index case of MEN-1. Manifestations include primary hyperparathyroidism; non-functioning pituitary adenoma with mild hyperprolactinemia, galactorrhea and secondary amenorrhea; hypergastrinemia; 2 cm pancreatic mass consistent with neuroendocrine tumor; and bilateral adrenal masses with evidence of mild hypercortisolism.

She was status-post a single-gland parathyroidectomy that was not curative of hyperparathyroidism. Hyperprolactinemia was controlled with bromocriptine.

Baseline octreotide scan revealed a large pelvic mass. CT confirmed an 8.7 cm right adnexal mass with foci of fat, soft tissue density and calcification consistent with teratoma. MRI and pelvic sonogram findings were similar. Germline MEN1 gene analysis demonstrated heterozygosity for a novel c.219_220delCG mutation. The deletion predicts a frameshift starting with codon Glycine 74, changing the amino acid to proline, and creating a premature stop codon 42 residues downstream, resulting in nonsense-mediated mRNA decay or premature protein truncation.

She underwent right salpingo-oophorectomy and removal of the adnexal mass. Operative findings were consistent with a dermoid cyst. Pathology demonstrated both benign dermoid cyst and neuroendocrine tumor consistent with carcinoid. Neuroendocrine tumor cells were positive for synaptophysin and chromogranin. DNA analysis revealed LOH at the MEN1 locus.

Conclusion: This case demonstrates a neuroendocrine tumor of the ovary with LOH at the MEN1 gene locus, a finding that is commonly seen in foregut carcinoids in MEN-1. Neuroendocrine tumor of the ovary has not been previously reported in a patient with MEN-1, either alone or in association with teratoma. This finding is perhaps related to the novel MEN1 gene mutation in this patient.

Nothing to Disclose: JMS, WFS, SJM

Pub # P3-269

Session Information POSTER SESSION: CLINICAL/TRANSLATIONAL - Neuroendocrine Tumors (1:30 PM-3:30 PM)

Title Hypoglycemia in Patients Presenting with Hepatic Somatostatin-Expressing Neuroendocrine Carcinomas

Author String P Wiesli, A Perren, M Brandle, C Schmid
Kantonsspital Frauenfeld, Frauenfeld, Switzerland; Universit[au]tsspital Bern, Bern, Switzerland;
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Body **Background:** Somatostatin-secreting neuroendocrine tumors may present with hypoglycemia. As opposed to pancreatic islet carcinomas with excessive insulin secretion from the pancreas and liver metastasis, the mechanism of hypoglycemia in patients with neuroendocrine liver tumors is less clear. We describe two such patients, presenting with malignant somatostatinomas.

Patients and Results: A 76-year-old man had multiple hepatic tumor masses, in retrospect (postmortem) malignant somatostatinoma of the pancreas with prominent liver metastasis. Immunohistochemical staining of tumor cells was positive for somatostatin in the liver (biopsy) and pancreas (at autopsy), and serum levels of somatostatin were markedly increased. The patient experienced hyperinsulinemic hypoglycemia exclusively in the postprandial period and following oral (but not iv) glucose. Selective arterial calcium stimulation with hepatic venous sampling (ASVS) showed no increase in insulin concentrations after stimulating the proper hepatic artery supplying the the bulk of the tumor but a positive response to calcium injection into all arteries supplying the pancreas. Liver artery embolization resulted in transient relief from postprandial hypoglycemia. Postmortem analysis of the pancreas confirmed islet hyperplasia (nesidioblastosis) throughout the pancreas. A 56-year-old woman presented with a neuroendocrine tumor of 10cm in diameter in the left hepatic lobe. Hyperinsulinemic hypoglycemia occurred in the fasting state. ASVS revealed no increase in insulin or somatostatin following calcium injection into the arteries supplying the pancreas but a marked increase in both hormones following calcium injection into the left hepatic artery. After resection of the tumor, liver tumor disease recurred but the patient remained free of dysglycemia for the subsequent 5 years (up to this writing). Immunohistochemical staining of the liver tumor and a lymph node metastasis was positive for both insulin and somatostatin.

Conclusions: In these two patients with malignant somatostatinoma, pancreatic islet hyperplasia and co-secretion of insulin (in addition to somatostatin) from hepatic tumor cells, respectively, have been characterized as completely distinct mechanisms of hypoglycemia at the functional and morphological level.

Nothing to Disclose: PW, AP, MB, CS

Pub # P3-270

Session Information POSTER SESSION: CLINICAL - Diverse Issues in Acromegaly (1:30 PM-3:30 PM)

Title Should Patients with Diabetes Be Routinely Screened for Acromegaly?

Author String G Thanabalasingham, M Selwood, AJ Farmer, MI McCarthy, JH Wass, KR Owen
University of Oxford, Oxford, UK; Churchill Hospital, Oxford, UK; University of Oxford, Oxford, UK;
University of Oxford, Oxford, UK

Body

Aims: The study aimed to evaluate the frequency of unrecognised acromegaly presenting as diabetes to assess the value of routine biochemical screening for acromegaly in these patients.

Methods: Serum insulin-like growth factor-1 (IGF-1) was measured in 215 patients with diabetes (of whom 159 have type 2 diabetes). No subjects were previously suspected to have pituitary disease including acromegaly. Serum IGF-1 values above the gender-specific and age-adjusted normal range were repeated along with the measurement of growth hormone (GH) during an oral glucose tolerance test (OGTT).

Results: In the patient group mean age at sampling (\pm SD) was 50.4 ± 12.7 years, HbA1c was $7.9 \pm 1.4\%$, and BMI was 31.6 ± 7.2 kg/m². Sixty percent of subjects were male and 67% were treated with insulin. Mean IGF-1 levels (\pm SD) were 15.4 ± 7.8 nmol/L. Three subjects (1.4%) were found to have elevated IGF-1 levels, and 2 attended for further assessment. Patient 1 had mild features of acromegaly and repeat IGF-1 level was markedly elevated at 85.7 nmol/L (normal range 10.5-34.7 nmol/L). During OGTT, GH levels paradoxically rose; basal GH 2.5 mcg/L and nadir GH 3.0 mcg/L. A dynamic MRI pituitary with gadolinium detected a microadenoma. Transphenoidal surgical resection achieved biochemical cure and improved glycaemic control. Immunohistochemistry confirmed a somatotroph pituitary adenoma. Patient 2 did not have any clinical features suggestive of acromegaly with only marginally raised IGF-1 levels at 37.7 nmol/L (range 10.5-34.7 nmol/L). GH levels remained suppressed during OGTT; basal GH 0.4 mcg/L and nadir GH 0.5 mcg/L. This patient remains under regular medical review with no change in the clinical picture.

Conclusion: This study found 0.5% prevalence of acromegaly in diabetes patients. The study does not support routine biochemical screening but emphasises that the endocrinopathies should be considered within the differential of diabetes aetiology and those with suggestive clinical phenotypes should undergo biochemical investigation. The study confirms previous reports that acromegaly is under-recognised as this patient's phenotype consistent with acromegaly had been overlooked despite regular medical review for diabetes.

Nothing to Disclose: GT, MS, AJF, MIM, JAHW, KRO

Pub # P3-271

Session Information POSTER SESSION: CLINICAL - Diverse Issues in Acromegaly (1:30 PM-3:30 PM)

Title Analysis of GH Response to Oral Glucose Tolerance Test in Acromegalic and Non-Acromegalic Individuals

Author String LA Naves, AO Hoff, SS Costa, LF Abdalla, CM Ferrer, L Monte, M Teles, RP Biscolla, JG Vieira
University of Brasilia, Brasilia, Brazil; Laboratorio Fleury, Brasilia, Brazil; Laboratorio Sabin, Brasilia, Brazil

Body **Background:** Acromegaly is a rare disorder that may need multiple therapeutic interventions to achieve disease control. The last decade has been marked by progressive improvement in the sensitivity of GH assays which can better delineate biochemical remission. Discrepancies between suppressed GH and basal IGF-1 levels may represent a disruption on the control of the somatotrophic axis and the need of functional tests as Oral Glucose Tolerance Test (OGTT) has raised questions regarding the most appropriate cut-off level that truly reflects remission.

Objective: To assess GH response to OGTT in treated and untreated acromegalics compared to normal controls. **Methods:** We investigated 31 patients with acromegaly (15 females and 16 males), ages 40.8 ± 12.6 years and 31 non-acromegalic patients (9 males and 22 females) ages 44 ± 13.49 years. IGF-I was measured at the basal level and GH and glucose at basal, 30, 60, 90 and 120 minutes after a 75g glucose overload. Serum GH was measured by the Beckman Access DXL assay (Beckman Coulter UK Ltd, High Wycombe, UK) and serum IGF-1 by the Immulite 2000 assay; Diagnostic Products Corp., Los Angeles, CA). A GH nadir < 0.4 ng/mL and/or an IGF-1 normal for age and sex were considered criteria for biochemical remission. **Results:** The acromegaly group was composed by 6 recently diagnosed patients (untreated) and 27 treated patients (under treatment). From the treated group, 10 patients achieved criteria for biochemical remission (controlled). The mean GH nadir level was significantly higher in controlled patients compared to non-acromegalic patients ($0.298 \text{ ng/mL} \pm 0.057$ Vs. 0.057 ± 0.05 , $P < 0.0001$) while the mean basal IGF-1 level was not significantly different between the 2 groups ($237.6 \text{ mcg/L} \pm 152.3$ in controlled acromegalics Vs. $184.32 \text{ mcg/L} \pm 71.4$, $P = 0.595$). **Discussion and Conclusion:** GH nadir levels after glucose suppression were significantly lower in healthy subjects than in controlled acromegalic patients ($p < 0.0001$). These findings suggest that despite attaining biochemical remission, normal somatotrophic physiology is not completely recovered.

Sources of Research Support: CNPq, FINATEC, Laboratorio Fleury, Laboratorio Sabin, Brazil.

Nothing to Disclose: LAN, AOH, SSC, LFA, CMF, LM, MT, RPB, JGV

Pub # P3-272
Session Information POSTER SESSION: CLINICAL - Diverse Issues in Acromegaly (1:30 PM-3:30 PM)
Title Features of Glucose Metabolism in Acromegalic Patients in Active Stage
Author String A Dreval, I Trigoloso, A Vinogradova, I Ilovayskaya
Moscow Regional Scientific Research Clinical Institute, Moscow, Russian Federation
Body Abstract Embargoed.
Nothing to Disclose: AD, IT, AV, II

Pub # P3-273

Session Information POSTER SESSION: CLINICAL - Diverse Issues in Acromegaly (1:30 PM-3:30 PM)

Title Correlation of Serum IGF-I Level with Insulin Resistance in Patients with Acromegaly

Author String H Koshiyama, Y Kawasaki, Y Iwasaki, K Mori, S Honjo, Y Hamamoto, H Ikeda, K Nomura, Y Wada, Y Takahasi, J Takahashi
Medical Research Institute KITANO Hospital, Osaka, Japan; Medical Research Institute KITANO Hospital, Osaka, Japan

Body We investigated the relationship of serum growth hormone (GH) and insulin-like growth factor-1 (IGF-1) to glucose tolerance in patients with acromegaly before and shortly after the operation. The study included a total of 14 patients with acromegaly (mean age 45 years; 6 women and 8 men) who received transsphenoidal surgery in our hospital from 2006 to 2009. All patients received 75g OGTT before and after surgery (mean \pm SD 10.4 \pm 2.8 days after surgery). Homeostasis model assessment of insulin resistance (HOMA-R) and insulinogenic index (IG) were calculated. Serum levels of GH and IGF-1 were determined before and after surgery and their correlation with HOMA-R was assessed. Before surgery, 9 patients had impaired glucose tolerance (IGT) and one patient had diabetes mellitus (DM) patterns; their fasting plasma glucose (FPG) level was 98.6 \pm 11.2 mg/dl and HOMA-R was 2.07 \pm 1.18. Postoperatively, the number of subjects who showed IGT pattern was decreased to four. Both FPG and HOMA-R significantly decreased (92.7 \pm 13.9 mg/dl, 1.49 \pm 1.06), whereas IG showed no significant change. HOMA-R showed a significantly positive correlation with serum IGF-1 levels both before and after surgery ($r=0.81$ and 0.57 , respectively), but not with serum GH levels. The differences in HOMA-R between before and after surgery also showed a significantly positive correlation with those in IGF-1 concentrations. These results suggest that serum IGF-level is well correlated with insulin resistance in patients with acromegaly, and that insulin resistance shows an improvement in early phase after surgery.

Nothing to Disclose: HK, YK, YI, KM, SH, YH, HI, KN, YW, YT, JT

Pub # P3-274

Session Information POSTER SESSION: CLINICAL - Diverse Issues in Acromegaly (1:30 PM-3:30 PM)

Title Mechanism and Management of Hyperglycemia Associated with Pasireotide: Results from Studies in Healthy Volunteers

Author String RR Henry, S Mudaliar, K Wetli-Hermosillo, M Ligueros-Saylan, S Chenji, G Golor
University of California at San Diego and VA San Diego Healthcare System, San Diego, CA; Oncology Business Unit, Novartis Pharma AG, Basel, Switzerland; Novartis Pharmaceuticals Corporation, Florham Park, NJ; Novartis Healthcare Pvt Ltd, Hyderabad, India; Parexel International GmbH, Berlin, Germany

Body

Introduction: The multireceptor-targeted somatostatin analogue pasireotide has high affinity for sst₅; this receptor may play an important role in glucose metabolism. Two studies in healthy male volunteers evaluated the mechanism and management of hyperglycemia associated with pasireotide.

Methods: A randomized, Phase II study assessed insulin secretion and glucose metabolism during 8 days of pasireotide 600 or 900[μg] sc bid (n=19 for both) with oral glucose tolerance test (OGTT), hyperglycemic clamp (HC) with arginine stimulation, and hyperinsulinemic-euglycemic clamp (HIEC). A randomized, 1-week, five-arm Phase I study evaluated glucose metabolism following OGTT with pasireotide 600[μg] sc bid co-administered with four classes of antihyperglycemic drugs (metformin, nateglinide, vildagliptin or liraglutide) or pasireotide alone (n=18 each).

Results: Following OGTT (Phase II study), significant decreases in mean plasma insulin AUC ($P<0.001$) and increases in mean plasma glucose AUC ($P<0.001$) were observed during treatment in both dose groups. Following HC, final plasma insulin levels were ~50% those at baseline; decreases in AUC were significant ($P<0.001$) in both dose groups. Significant decreases ($P<0.01$) in glucagon-like peptide-1 (GLP-1) and gastric inhibitory peptide (GIP) AUC were observed. Following HIEC there were no statistically significant changes in basal endogenous glucose production or glucose disposal rate (ie insulin sensitivity). The plasma glucose AUC_{0-4h} on day 7 (Phase I study) decreased by a mean of 2%, 10%, 15% and 29%, respectively, when pasireotide was co-administered with metformin, nateglinide, vildagliptin and liraglutide, compared with pasireotide alone. Treatment was generally well tolerated in these groups.

Conclusions: Hyperglycemia associated with pasireotide is related to decreases in insulin secretion, with no changes in insulin sensitivity. Pasireotide also significantly decreased incretin hormone response. The mechanism of action of GLP-1 analogues (eg liraglutide), DPP-4 inhibitors (eg vildagliptin) and insulin secretagogues (eg nateglinide) can effectively address the hyperglycemia on pasireotide therapy.

Sources of Research Support: Novartis Pharma AG.

Disclosures: ML-S: Employee, Novartis Pharmaceuticals. SC: Employee, Novartis Pharmaceuticals. Nothing to Disclose: RRH, SYM, KW-H, GG

Pub # P3-275

Session Information POSTER SESSION: CLINICAL - Diverse Issues in Acromegaly (1:30 PM-3:30 PM)

Title Gonadotropin Responses with LHRH Stimulation Were Influenced by Tumor Size in Korean Acromegalics

Author String S-W Kim, SO Chin, S Oh
College of Medicine, Kyung Hee University, Seoul, Korea

Body Acromegaly had various physiologic condition with its disease duration, tumor size or genetic background. Gonadal dysfunction of female acromegalics were originated from tumor mass effect and/or hyperprolactinemia. We reviewed sixty-three Acromegalics (M:F=28:35) for the last five years, who had taken oGTT (75 gram of glucose) test, LHRH (100 [micro]g) with checking GH and insulin before and after treatment in Kyung Hee University Medical Center, Seoul, South Korea. Among them, we selected 39 macroadenoma (M:F=21:18) patients for further analysis. Serum LH and FSH had been checked at before and 30 min. after LHRH injection. Average age of enrolled patient was 43±10 years old. We divided patients with basal GH levels by 40 [micro]g/L, group 1 (26) was defined as over 40 [micro]g/L of basal GH. Group 2 was below 40 [micro]g/L of basal GH. Group 2 was higher level of LH, and FSH levels after LHRH stimulation. These effects were due to small tumor size, because macroadenoma patients showed much low FSH levels and delayed stimulated LH levels. We concluded major factor influenced to gonadal function of acromegalics was large tumor size, not high basal GH levels.

Micha[euml]l Grynberg et al., J Clin Endocrinol Metab 2010;95:4518-4525

Nothing to Disclose: S-WK, SOC, SO

Pub # P3-276

Session Information POSTER SESSION: CLINICAL - Diverse Issues in Acromegaly (1:30 PM-3:30 PM)

Title Body Composition, Cardiovascular Risk Markers and Disease Control in 120 Patients with Acromegaly

Author String TJ Reid, CM Reyes-Vidal, IM Conwell, CA Navarro, EB Geer, KD Post, JN Bruce, PU Freda
Columbia University, College of Physicians & Surgeons, New York, NY; Mount Sinai School of Medicine, New York, NY; Mount Sinai School of Medicine, New York, NY; Columbia University, College of Physicians & Surgeons, New York, NY

Body Active acromegaly is associated with increased mortality, which has been attributed largely to cardiovascular (CV) disease. However, markers of CV risk in acromegaly do not all follow the pattern characteristic of increased risk in the general population.

In order to further elucidate determinants of CV risk in acromegaly before and after treatment we examined biochemical [growth hormone (GH), insulin-like growth factor-I (IGF-I), insulin, glucose] and CV risk [C-reactive protein (CRP) and homocysteine] markers and body composition by dual-energy X-ray absorptiometry (DXA) in a cross-sectional study of acromegaly patients across a spectrum of disease. Hormones were measured by chemiluminescent immunometric assay (Siemens, DPC). IGF-I levels were analyzed as a % of the upper limit of normal (% ULN) for age and GH as the nadir during a 100 gm OGTT. Data are expressed as mean \pm SE or median (interquartile range), group differences tested by Mann-Whitney U-test and correlations by Spearman rank correlation.

We studied 120 patients with acromegaly; 65% had active disease (IGF-I > ULN; 44% newly diagnosed and 56% with persistent disease after surgery) and 35% were in remission (normal IGF-I) after surgery. The cohort was 61% male and 39% female with a mean age of 48.4 ± 1.1 yrs (range 18-78 yrs) and BMI 30 ± 0.5 kg/m².

The active vs. remission group had lower levels of CRP [0.30 (0.30-0.62) vs. 0.96 (0.35-2.54) mg/L (p=0.001)], homocysteine [7.88 (6.40-10.70) vs. 9.38 (8.14-11.53) [micro]mol/L (p=0.03)] and % total body fat (28.34 ± 1.31 vs. $36.66 \pm 1.42\%$, p=0.0001), but higher HOMA-IR (3.66 ± 0.50 vs. 1.82 ± 0.21 , p=0.005). CRP correlated with homocysteine (p=0.0008), BMI (p=0.0007), % total body fat (p < 0.0001) and waist-hip ratio (p=0.026), inversely with % ULN IGF-I (p=0.0006) and not with HOMA-IR (p=0.26). Homocysteine correlated with % total body fat (in males only, p=0.023), inversely with % ULN IGF-I (p=0.0011) and HOMA-IR (p=0.027) but not with BMI or waist-hip ratio. Nadir GH correlated inversely with % total body fat, CRP and homocysteine (p < 0.0001 for all).

These data show that biochemical markers correlate with features of CV risk in acromegaly, but remission is associated with higher CRP, homocysteine and % total body fat, more consistent with increased risk. The long-term implications of modern criteria of disease control on CV risk in treated acromegaly patients warrants further study.

Sources of Research Support: NIH grants DK064720, DK064720-06A1W1 and DK073040.

Nothing to Disclose: TJR, CMR-V, IMC, CAN, EBG, KDP, JNB, PUF

Pub # P3-277

Session Information POSTER SESSION: CLINICAL - Diverse Issues in Acromegaly (1:30 PM-3:30 PM)

Title sRANKL/OPG Ratio and Relation with Bone Resorption in Patients with Acromegaly and Prolactinoma

Author String F Firat, N Cinar, K Aydin, S Akin, S Dagdelen, T Erbas
Hacettepe University Medical School, Ankara, Turkey

Body It is known that osteoprotegerin (OPG) and RANKL play an important role in bone remodelling. However, no studies have investigated the level of sRANKL and OPG in acromegaly and prolactinoma so far. Aim of this study is to evaluate effect of hyperprolactinemia and increased levels of IGF-I on bone resorption and their relation with RANKL/OPG ratio.

Subjects are grouped as acromegaly (n:31), prolactinoma (n:28) and control group (n:33). OPG, sRANKL and bone turnover markers [bone alkaline phosphatase (BALP), osteocalcin (OC), type1 collagen (COL1), C telopeptid (CTX) and urine deoxypyridinoline (DPD)] levels were evaluated. BMD was measured by DEXA. Statistical analysis was performed on SPSS 15.0. Significance was accepted at $p < 0.05$. The median OPG levels in acromegaly and prolactinoma were 13.71 and 11.57 pg/ml, respectively, which did not significantly differ from the controls (11.8 pg/ml). The median level of sRANKL were similar in the groups: acromegaly, 0.218 pmol/L; prolactinoma, 0.195 pmol/L; control, 0.148 pmol/L. Although the ratio of sRANKL/OPG (0.199) was decreased in control, no significant difference was observed between the groups (acromegaly, 0.336; prolactinoma, 0.376). While CTX level was increased in acromegaly and significantly differ from control ($p=0.013$), COL 1 level was significantly increased in both acromegaly and prolactinoma ($p=0.003$ and $p=0.018$, respectively). The median PTH level was increased (64 pg/mL) in acromegaly and significantly differ from prolactinoma (43 pg/mL) and control (44 pg/mL). A positive correlation between OPG and age in prolactinoma and control was detected ($r=0.524$, $p=0.004$ and $r=0.380$, $p=0.029$, respectively). A significant negative association is observed between OPG and IGF-I values after excluding age in prolactinoma ($r=-0.412$, $p=0.046$). OC and BALP were negatively associated with sRANKL level in acromegaly ($r=-0.384$, $p=0.036$, and $r=-0.528$, $p=0.003$). Neither levels of OPG nor sRANKL or sRANKL/OPG ratio correlated with BMDs.

RANKL level is associated with bone formation markers, rather than BMD values. Bone turnover is increased in acromegaly and negatively associated with sRANKL. IGF-I level is not associated with RANKL but negatively correlated with OPG. RANKL and OPG levels are not associated with prolactin level. Also, bone turnover is not high as acromegaly in prolactinoma group. However, our results may reflect conditions in the intact organism rather than isolated effects of prolactin and IGF-I.

Nothing to Disclose: FF, NC, KA, SA, SD, TE

Pub # P3-278

Session Information POSTER SESSION: CLINICAL - Diverse Issues in Acromegaly (1:30 PM-3:30 PM)

Title Fibrous Dysplasia with Acromegaly

Author String M Goyal, H Tiwana, A Lee
NuHealth/Nassau University Medical Center, East Meadow, NY

Body McCune-Albright Syndrome (MAS) is a triad of fibrous dysplasia of bone (97%), café-au-lait macules (85%) and precocious puberty (52%). It is due to mutation in the Gs protein α subunit leading to cAMP overproduction. Hyperactive endocrinopathies associated with MAS include Cushing's Syndrome (50%), growth hormone and prolactin excess (20-30%), and Hyperthyroidism. Renal phosphate wasting and benign or malignant thyroid nodules have also been described. The presence of fibrous dysplasia and at least one of the hyperactive endocrinopathies is sufficient for diagnosis.

We evaluated a 30 year old Hispanic female who presented with oligomenorrhea and intermittent galactorrhea. History included a benign solitary thyroid nodule, Carpal Tunnel Syndrome, and headache. The patient noted an increased shoe size over the preceding 1 to 2 years. Thelarche occurred at age 13, menarche at age 14. Physical examination revealed bony prominences of the skull, a thyroid nodule and galactorrhea. Café-au-lait lesions were not present. Abnormal laboratory tests included elevated alkaline phosphatase (up to 230 U/L; normal 39-117), growth hormone (26.4 ng/ml; normal <10), IGF-1 (622 ng/ml; normal 64-334) and prolactin (71 ng/ml; normal 3-18.6). Bone scan showed increased isotope uptake in facial and frontal skull bones as well as the forearms and legs. X-rays showed diffuse thickening of the cortex with multiple areas of lucency suggestive of fibrous dysplasia in the skull and femur. MRI documented a 2.3 cm. pituitary macroadenoma with some compression of the optic chiasm

MAS with Acromegaly was presumptively diagnosed and the patient underwent partial transphenoidal resection followed by somatostatin therapy. Seven months later IGF-1 was improved, but still elevated (376 ng/ml). Bone specific alkaline phosphatase was high (59 [micro]g/L; 5.3-19.5).

Somatostatin analogues may be only partially effective treating Acromegaly in MAS. Pegvisomant, a GH receptor antagonist, may be more effective in lowering IGF-1 levels. Radiation therapy is generally avoided in MAS because of the risk of development of malignancy in fibrous dysplasia tissue. Hence, it is important to consider MAS (and fibrous dysplasia) in all patients with Acromegaly.

In conclusion, we present a case, with a review of the literature, of McCune-Albright Syndrome with Acromegaly but without café-au-lait lesions or precocious puberty, who presented as an adult with galactorrhea and oligomenorrhea.

Nothing to Disclose: MG, HT, AL

Pub # P3-279

Session Information POSTER SESSION: CLINICAL - Diverse Issues in Acromegaly (1:30 PM-3:30 PM)

Title Assessment of P300 Auditory Event-Related Potentials in Acromegaly

Author String A Taslipinar, O Oz, M Yucel, E Bolu, UH Ulas, H Akgun, Y Kutukcu, Z Odabasi, M Kutlu
Department of Endocrinology and Metabolism, Ankara, Turkey; Department of Neurology, Ankara, Turkey

Body **BACKGROUND:** Neurophysiological techniques have provided some of the most direct evidence for assessing central nervous system functions. Event related potentials (ERPs) have been widely used to investigate the mechanisms underlying cognitive functions. There is limited information in the literature concerning ERPs' parameters (duration and amplitude) and their correlations with serum GH and IGF-1 levels in acromegaly.
AIM: The aim of this study is to evaluate the cognitive function in Mini Mental Status Scale (MMSS) matched (normal MMSS) patients with acromegaly and compare with control subjects and assess the correlation between cognitive functions and IGF-1 levels.
SUBJECTS AND METHODS: The study comprised 15 male patients with Acromegaly and 30 age, education and sex-matched healthy controls. Patients and controls underwent electroencephalogram activity measurement from the electrode placed at the midline central (Cz) zone. ERPs were obtained. Latency and amplitude measurements of the patients were compared with controls. Correlation between latency and amplitude values of P300 and serum levels of IGF-1 were analyzed.
RESULTS: The data obtained from Cz electrode site showed significantly prolonged P300 latencies ($p < 0.001$) and significantly reduced P300 amplitudes ($p = 0.007$) in acromegalics when compared with controls. Serum IGF-1 levels were positively correlated with latency ($r: 0.688, p: 0.005$) and negatively correlated with amplitudes ($r: -0.670, p: 0.006$) in patient group
CONCLUSIONS: There might be an early electrophysiological evidence of cognitive impairment in patients with acromegaly by means of P300 measurements. IGF-1 seems to play a critical role in cognitive impairment of these individuals.

- 1) The Diagnosis and Treatment of Acromegaly Laurence Katznelson, MD The Endocrinologist Volume 13, Number 5, October 2003
- 2) Taslipinar A, Bolu E, Kebapcilar L, Sahin M, Uckaya G, Kutlu M. Insulin-like growth factor-1 is essential to the increased mortality caused by excess growth hormone: a case of thyroid cancer and non-Hodgkin's lymphoma in a patient with pituitary acromegaly. Med Oncol 2009, 26(1); 62-66
- 3) Deijen JB, de Boer H, Blok GJ, van der Veen EA. Cognitive impairments and mood disturbances in growth hormone deficient men. Psychoneuroendocrinology 1996, 21 313-322.

Nothing to Disclose: AT, OO, MY, EB, UHU, HA, YK, ZO, MK

Pub # P3-280

Session Information POSTER SESSION: CLINICAL - Diverse Issues in Acromegaly (1:30 PM-3:30 PM)

Title Normal Sensorineural Hearing, and Peripheral/Central Nervous Auditory System Transmission in Acromegalic Patients under Treatment (Evaluated by Brainstem Auditory Evoked Potentials and Pure Tone Audiometry)

Author String MA Carvalho, RM Jr, MR Freitas, MF Albano, VO Fernandes, AR Quidute, L Vilar, R Montengro, ATB Mendonca
Faculty of Medicine, Federal University of Ceará, Fortaleza, Brazil; Faculty of Medicine, Federal University of Pernambuco, Recife, Brazil

Body **Objective:** To assess the function of the central and peripheral nervous systems in patients with active and controlled acromegaly by using brainstem auditory evoked potentials (BAEP); and to evaluate the prevalence of sensorineural hearing loss (SHL) in these two groups.
Subjects and methods: This is a cross-sectional study carried out in 36 acromegalic patients followed at a University Hospital in the northeast of Brazil, a tertiary referral center. All patients have been treated with somatostatin analogs (octreotide) 29 (80,5%) alone or with its association with cabergoline 7 (19,5%). Of these, 25 (69,4%) were previously submitted to transfenoidal surgery. An audiological assessment consisting in pure-tone audiometry (PTA) and the determination of BAEP (bilateral peak I latency and interpeaks I-III, III-V and I-V) were performed. The patients were divided in two groups, with active (A) and controlled (C) disease according to the GH and IGF-1 levels, using the Endocrine Society criteria (2010).
Results: Among these patients, 15 had controlled and 21 active disease at the time of the evaluation. Both groups have similar prevalence of diabetes and impaired glucose tolerance (46,6% in the C group vs. 57,1% in the A group), as well as of hypopituitarism (20% in the C group vs. 33,3% in the A group). There was no difference in the prevalence of SHL between groups (46,6% in the C group vs. 33,3% in the A group). No statistically significant differences were also observed between groups regarding the components of BAEP. Furthermore, no correlation was observed between BAEP components, and GH or IGF-1 levels.
Conclusions: The effects of excess GH and IGF-1 secretion in acromegalic patients seems to do not affect their sensorineural hearing nor peripheral or central nervous auditory system transmission, at least in patients who are under treatment.

Nothing to Disclose: MAC, RMJ, MRF, MFA, VOF, ARQ, LV, RM, ATBM

Pub # P3-281

Session Information POSTER SESSION: CLINICAL - Diverse Issues in Acromegaly (1:30 PM-3:30 PM)

Title Malignant Tumors in Patients with Acromegaly

Author String HJ Choi, AR Khang, JH An, ES Hong, KW Kim, SW Kim, CS Shin, SY Kim
Seoul National University College of Medicine, Seoul, Republic of Korea

Body

Background: An increased incidence of malignancy in acromegaly has been reported in several studies, but a direct cause-effect association between acromegaly and malignancy has not been proved. The aim of this study was to investigate the characteristics of acromegalic patients who developed cancer.

Method: Two hundred-twenty five patients (116 female, 109 male) with acromegaly were followed and treated at Seoul National University Hospital from January 1995 to September 2009.

Result: Malignancy was detected in 16 (7.1%, Male 7, Female 9) patients. In female patients, colon cancer was found in 3 patients, thyroid cancer in 2, leukemia in 2, lung cancer in 1, and pancreatic cancer in 1 patient. In male patients, stomach cancer was found in 3 patients, colon cancer in 1, pancreatic cancer in 1, prostate cancer in 1 and thyroid cancer in 1 patient. In patients without cancer, the age of acromegaly at diagnosis was 42 ± 12 years old and the duration of follow-up was 7.5 ± 6.6 years. In patients with cancer, the age of acromegaly at diagnosis was 51 ± 10 years old, the age of cancer at diagnosis was 56 ± 11 years old, and the duration from diagnosis of acromegaly to malignancy was 5.3 ± 5.4 years. The gender, family history of cancer, duration of follow-up, initial and last IGF-I and GH levels, initial tumor size and presence of residual tumor were not associated with cancer development.

Conclusion: The overall incidence of malignancy in patients with acromegaly was 16 of 225 patients (7.1%). There were no differences between With-Cancer group and Without-Cancer group in duration of follow-up, IGF-1, GH level, tumor size (at diagnosis and last) and presence of residual tumor except age at diagnosis of acromegaly.

Nothing to Disclose: HJC, ARK, JHA, ESH, KWK, SWK, CSS, SYK

Pub # P3-282

Session Information POSTER SESSION: CLINICAL - Diverse Issues in Acromegaly (1:30 PM-3:30 PM)

Title Pheochromocytoma and Acromegaly: A Rare but Relevant Co-Existence

Author String S Sharma, M Gurnell, DG Morris
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Body **Introduction:** Acromegaly and Pheochromocytoma are both rare diseases but their co-existence is even rare with estimated probability in the order of 1.6 per 1 billion. Various hypotheses quoted in literature include suggestions that this occurrence may be a variant of multiple endocrine neoplasia (MEN) syndrome despite the absence of any inheritance pattern ¹. Another causal hypothesis linked them to pituitary somatotroph hyperplasia secondary to ectopic production of growth hormone-releasing hormone (GHRH) by the pheochromocytoma ². We describe a patient with a growth hormone (GH)-secreting pituitary adenoma and a pheochromocytoma whose onset was spread over 19 years.

Clinical Case: We present a 40 year old patient who was diagnosed and operated for a right sided pheochromocytoma at the age of 20. Thereafter, she had a complete recovery and remained both clinically and biochemically stable. Genetic screening for RET proto-oncogene was negative.

In 2006, she started to notice increasing acral enlargement and in 2009, was found to have bi-temporal visual field defects when she complained of visual problems. MRI revealed a large 4cm macroadenoma stretching up the left optic chiasm on the left and invading the cavernous sinus on the right. Further biochemical testing confirmed the diagnosis of Acromegaly. Initial medical management with Somatostatin analogues showed partial response in terms of visual field symptoms and reduction in pituitary size. She was re-investigated for her previous pheochromocytoma and repeat catecholamine assays showed contradictory outcomes. Although a MIBG scan demonstrated increased tracer uptake in the right adrenal bed raising suspicion of residual disease, CT imaging showed no cross-sectional evidence of dormant tissue in this location. Screening for SDH complex and VHL gene were negative. She was pre-treated with Doxazocin and underwent a trans-sphenoidal surgery in 2010 with histology consistent with GH secreting adenoma. Presently, she is on 90 mg Lanreotide Autogel 4-weekly and biochemical control of the Acromegaly remains sub-optimal pending pituitary radiotherapy.

Conclusion: In a patient with acromegaly and hypertension, the clinician should be aware of this possible co-existence of dual endocrinopathies. Further genetic studies to determine loss of tumor suppressor genes or activation of proto-oncogenes are sought for and also to isolate a putative growth factor that would stimulate the development of both neoplasms.

(1) Anderson RJ et al., Acromegaly and pituitary adenoma with pheochromocytoma: a variant of multiple endocrine neoplasia. Clin Endocrinol (Oxf). 1981;14:605-612.

(2) Saito H et al., Demonstration of biological activity of a growth hormone-releasing hormone-like substance produced by a pheochromocytoma. Acta Endocrinol (Copenh). 1993;129:246-250.

Nothing to Disclose: SS, MG, DGM

Pub # P3-283

Session Information POSTER SESSION: CLINICAL - Diverse Issues in Acromegaly (1:30 PM-3:30 PM)

Title Endoscopic Surgery, a New Standard for Surgical Treatment of Acromegaly: Experience in 80 Patients Treated by a Single Neurosurgeon

Author String F Castinetti, V Leca, T Graillon, I Morange, F Albarel, R Gras, T Brue, H Dufour
La Timone Hospital, Marseille, France; La Timone Hospital, Marseille, France; La Timone Hospital, Marseille, France

Body The first line treatment of acromegaly is transsphenoidal surgery. It induces remission in 50-80% cases, depending on adenoma size and degree of invasiveness, initial hormonal levels and neurosurgeon's experience. Until recently, surgical procedure was based on a microscopic transphenoidal approach. We report our experience of 80 consecutive patients with acromegaly treated with an endoscopic endonasal surgical approach by a single neurosurgeon in La Timone Hospital, Marseille, France.
Eight patients who had a follow-up inferior to 3 months were excluded. Seventy-two patients with a mean follow-up of 23 months were evaluated. At the last timepoint, 65% (n=47) were in remission, defined by strict criteria (GH inferior to 0.4 ng/ml after blood glucose load and normalized IGF-1 level). Five patients, who had clinical improvement, normal IGF1 levels but increased GH levels, were classified as having an unsuccessful surgery (follow-up, 33 months) despite the fact they were given no additional treatment. Out of 19 patients with persistently high GH and IGF-1 levels at last follow-up, ten were controlled by medical treatments and/or radiotherapy, whereas nine remained uncontrolled (follow-up: 23 months).
Endoscopic surgery induced remission in at least 75% of cases for enclosed adenomas (microadenomas -84% remission- and macroadenomas -77% remission) and invasive microadenomas (75% remission), and less than 50% for invasive macroadenomas. Persistent adverse effects were observed in 8% of cases: they included diabetes insipidus (n=3), worsening of pituitary function (n=2) and hyposmia (n=1). No cerebrospinal leak or meningitis was observed.
To conclude, our study is one of the largest series published to date performed by a single neurosurgeon. Endoscopic approach is at least as effective as microscopic approach, with a decreased rate of permanent adverse effects, a similar cure rate when compared with the best endocrinological results already published. Endoscopy leads to a better operative comfort for the surgeon and better post-operative suite for the patient. Endoscopic technique without nasal retractor allows a better view of the lateral compartment of the sella, which can make resection of enclosed macroadenomas easier. It could also be useful for resection of tumoral extension in the inner compartment of the cavernous sinus. It should thus be the preferred surgical procedure for the treatment of acromegaly.
*First 2 authors contributed equally to this work.

Nothing to Disclose: FC, VL, TG, IM, FA, RG, TB, HD

Pub # P3-284

Session Information POSTER SESSION: CLINICAL - Diverse Issues in Acromegaly (1:30 PM-3:30 PM)

Title Low Incidence of Adrenal Insufficiency after Transsphenoidal Surgery in Patients with Acromegaly: A Long-Term Follow-Up Study

Author String AG Burgers, NE Kokshoorn, AM Pereira, F Roelfsema, JWA Smit, NR Biermasz, JA Romijn
Leiden University Medical Center, Leiden, Netherlands

Body
Background: The long-term prevalence of adrenal insufficiency after transsphenoidal surgery for growth-hormone secreting pituitary adenomas was unknown. However, recently a single study reported a high prevalence of adrenal insufficiency after surgical and/or medical treatment without postoperative radiotherapy in acromegalic patients.
Aim: To assess the prevalence and incidence rate of adrenal insufficiency in consecutive patients during long-term follow-up after successful transsphenoidal surgery for acromegaly.
Methods: In 91 consecutive patients in remission after transsphenoidal surgery, we retrospectively reviewed insulin tolerance tests, CRH stimulation tests, metyrapone tests and ACTH stimulation tests used to assess corticotrope function.
Results: Early postoperatively, insufficient adrenal function was observed in 18% of patient (n=16), which was transient in 8 and irreversible in 8 other patients in the first year of postoperative follow-up. Therefore, after the first year, the prevalence of adrenal insufficiency was 9%. Late, new-onset adrenal insufficiency developed in only 3 patients, 13, 18 and 24 years postoperatively, resp. The incidence rate of late adrenal insufficiency after successful surgery was 2/1000 person years.
After long-term follow-up, with a mean duration of 17.7 ± 8.1 yr, the prevalence of secondary adrenal insufficiency was 12% in patients in remission after surgery for acromegaly.
Conclusion: The prevalence of adrenal insufficiency 1 year after surgery was 9%, whereas during prolonged follow-up the incidence rate of adrenal insufficiency was only 2/1000 person years in patients in remission after surgery. Therefore, development of late onset adrenal insufficiency is a very infrequent complication in patients with acromegaly in remission after transsphenoidal surgery only.

Nothing to Disclose: AGB, NEK, AMP, FR, JWAS, NRB, JAR

Pub # P3-285

Session Information POSTER SESSION: CLINICAL - Diverse Issues in Acromegaly (1:30 PM-3:30 PM)

Title Long-Term Follow-Up of Controlled Acromegalic Patients: Radiotherapy Is Associated with Impaired Subjective Health Status (SF-36)

Author String S Schindler, M Mohlig, N Kremenevskaya, M Buchfelder, C Schofl
Erlangen University Hospital, Erlangen, Germany; Erlangen University Hospital, Erlangen, Germany; Charité-University Medicine Berlin, Campus Benjamin Franklin, Berlin, Germany

Body
Introduction: Acromegaly is associated with significant comorbidities and reduced quality of life (QoL). Information about QoL and the subjective health status during long-term follow-up of acromegalic patients operated on a GH-secreting pituitary adenoma in Germany is scarce.
Methods: The SF-36 was completed by 126 patients (mean age 52.7±15.5y, 68 women) who had surgery for acromegaly between 1996 and 2006 at our institution and that had normal age- and sex-adjusted IGF-1 levels. Scores for the 8 domains of the SF-36 were calculated and expressed as age- and sex-specific z-scores based on a random representative sample from the German population. Sex, age, adenoma size (micro- or macroadenoma), radiotherapy (n = 27), medical therapy (n = 42), and hormone replacement (n = 57) were analyzed for an association with the 8 domains of the SF-36 by backward linear regression analyses.
Results: In the 126 acromegalic patients z-scores for physical functioning (PF), role functioning physical (RP), bodily pain (BP), general health perception (GH), vitality (VT), social functioning (SF), role functioning emotional (RE), and mental health (MH) were - 0,45 ± 1,37, - 0,42 ± 1,38, - 0,26 ± 1,15, - 0,39 ± 1,27, - 0,59 ± 1,38, - 0,49 ± 1,41, - 0,83 ± 1,64, - 0,59 ± 1,29, respectively. Backward linear regression analyses for the 8 dimensions revealed that radiotherapy was the only parameter remaining in the final model. Radiotherapy was associated with a worse score in any of the 8 dimensions of the SF-36.
Conclusion: Radiotherapy was the most important factor associated with reduced physical and mental well-being according to the SF-36 in controlled acromegalic patients during long-term postoperative follow-up.

Sources of Research Support: Grant from Novartis Pharma GmbH, Nuremberg, Germany.

Disclosures: CS: Investigator, Novartis Pharmaceuticals. Nothing to Disclose: SS, MM, NK, MB

Pub # P3-286

Session Information POSTER SESSION: CLINICAL - Diverse Issues in Acromegaly (1:30 PM-3:30 PM)

Title Gamma-Knife Radiosurgery in Acromegaly Patients

Author String FM Erdur, T Kilic, S Peker, O Celik, P Kadioglu
Cerrahpasa Medical School, University of Istanbul, Istanbul, Turkey; Marmara Medical School, University of Marmara, Istanbul, Turkey; Acibadem Medical School, Istanbul, Turkey

Body **Objective:** The aim of the study was to evaluate the efficacy and reliability of gamma-knife radiosurgery (GKR) in acromegalic patients.
Subjects and Methods: Twenty-two acromegalic patients treated with GKR and followed up at the Endocrinology-Metabolism Clinic of Cerrahpasa Medical School, the University of Istanbul were included in the study. The diagnosis of active disease was determined by the presence of clinical findings and failure to suppress nadir GH level less than 1 ng/dl during OGTT and as well as high levels of IGF-1 adjusted for age and gender. Patients who did not achieve remission after surgery and therefore received only octreotid-LAR (30-40 mg / 28 days) or octreotid-LAR (30-40 mg/day) and dopamine agonist (cabergoline 3mg/week, 50 mg/day bromocriptin) combination therapy were chosen for GKR. The medical data on activity of the illness and other pituitary functions, pituitary magnetic resonance imaging (MRI) and visual fields, before GKR and after the 6th, 12th, 24th, 36th, 48th and 60th months of GKR were collected from the hospital records.
Results: The median follow up duration of the patients after GKR was 60 [Interquartile Range (IQR): 24 - 60 months]. The remission rate was 54.5 % after the 60 months of follow-up. The median growth hormone (GH) level after the GKR in 60 months (0.99 ng/ml [IQR: 0.36 - 2.2]) was significantly lower than median GH level before GKR (5.65 ng/ml [IQR: 3.85 - 7.2]) (p=0.002). The median insulin-like growth factor-1 (IGF-1) level after the GKR in 60 months (221.5 ng/ml [IQR: 149 - 535]) was significantly lower than the median IGF-1 level before GKR (582.5 ng/ml [IQR: 515 - 655]) (p=0.008). Tumor growth was under control in twenty patients (95.2 %). Six patients (28.6 %) developed new-onset hypopituitarism.
In conclusion despite the low number of patients and short duration of follow-up periods this study has shown that GKR seems to be an efficient and a safe method for controlling the tumor growth, reducing the increased GH and IGF-1 levels, and increasing the rate of remission of acromegaly patients resistant to surgical and/or medical treatment methods, especially in countries with problems of social health insurance. Although few side-effects of GKR were noted, further studies with longer follow-up are needed to evaluate the efficiency and safety of GKR.

Nothing to Disclose: FME, TK, SP, OC, PK

Pub # P3-287

Session Information POSTER SESSION: CLINICAL - Diverse Issues in Acromegaly (1:30 PM-3:30 PM)

Title Effectiveness of Cabergoline Treatment in Cases of Uncontrolled Acromegaly

Author String YA Tutuncu, D Berker, S Isik, G Akbaba, U Ozuguz, FK Kucukler, S Guler
Ministry of Health, Ankara Numune Research and Training Hospital, Ankara, Turkey

Body

Objective: Today, somatostatin analogues are primary medical treatment of choice for acromegaly. Additional cabergoline (CAB) therapy may be useful in patients uncontrolled with somatostatin analogues. In this study, we aimed to evaluate the effectiveness of the addition of CAB treatment in patients with acromegaly uncontrolled on octreotide LAR (OCT) or lanreotide Autogel (LAN).

Methods: Twenty-six patients with active acromegaly while receiving OCT (n=16) or LAN (n=10) treatment were included in the study. Cabergoline was added as an initial dose of 0.5 mg / week. In the control visits CAB dosage increased up to maximum of 3.5 mg/week dose in patients without normalization of IGF-1 and GH levels. Dose escalation in 3 patients could not be performed because of gastrointestinal symptoms and in one patient because of vertigo. Patients were evaluated for humoral and tumoral control after 6 months of CAB addition.

Results: Eighteen females and 8 males with a mean age of 38.5 ± 12.0 years consisted the study group. Mean cabergoline dose was 3.1 ± 0.2 mg / week. Growth hormone, IGF, and the mean tumor volume was significantly decreased after cabergoline treatment (7.1 ± 7.9 [mu]/l vs 3.3 ± 3.8 [mu]/l, $p=0.001$; 634.9 ± 316.4 vs 417 ± 232.0 , $p=0.001$ and $3.5 \pm 6.9 \text{ cm}^3$ vs $2.8 \pm 4.4 \text{ cm}^3$, $p<0.001$, respectively). 34.6% of patients achieved normalization of IGF-1 levels, and GH levels were reduced below 2.5 [mu]/l in 52.6% of patients after addition of CAB treatment. Tumor shrinkage was observed in 61.6% of the patients [0-25% in 7 patients (% 26.9), 25-50% in 4 patients (%15.4) and $\geq 50\%$ in 5 patients (%19.2)]. At the end of 6 months 10 patients (38.5%) showed sufficient GH suppression. There was no difference in terms of effectiveness of CAB between OCT and LAN groups.

Conclusion: Combination of CAB with somatostatin analogs should be considered as an effective and relatively affordable additive treatment in acromegaly for patients who do not accept reoperation or do possess high risk for reoperation before considering treatment with GH-receptor antagonists.

Nothing to Disclose: YAT, DB, SI, GA, UO, FKK, SG

Pub # P3-288

Session Information POSTER SESSION: CLINICAL - Diverse Issues in Acromegaly (1:30 PM-3:30 PM)

Title Ki-67 as a Predictor of Response to Octreotide LAR in Acromegaly Independently of SSTR2 Expression

Author String LKJ de Pinho, LEA Wildemberg, LV Neto, CM Takiya, MR Gadelha
Federal University of Rio de Janeiro, Rio de Janeiro, Brazil; Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

Body Introduction: Somatostatin analogues (SA) are currently the mainstay in the medical treatment of acromegalic patients. This drug class results in disease control in approximately 50-60% of patients. Laboratorial and molecular data have been studied for prediction of response to SA, like acute octreotide test and gsp status, with conflicting results. The somatostatin receptor type 2 (SSTR2) expression is the most established predictor of response to SA. There is only one study evaluating Ki-67 labeling index (LI) as a predictor of response to SA, however, SSTR2 expression was not considered in this study (1).
Objective: To evaluate if Ki-67 LI is as a predictor of response to SA, independently of SSTR2 expression.
Methods: Acromegalic patients consecutively operated on and not cured by surgery were included in the study. Exclusion criteria consisted in a history of medical treatment with SA, dopamine agonists or GH antagonist before surgery, and previous radiotherapy for pituitary adenoma. Protein expression was analyzed by immunohistochemistry. Tumors were classified according to percentage of cell immunolabeled nuclei for Ki-67 and both membrane bound and intracytoplasmic immunopositivity for SSTR2. The SSTR2 expression was considered high when more than 25% of tumor cells were immunostained and a cutoff of 2.3% was considered for Ki-67 LI (2). Patients were considered controlled when they presented normal age-matched IGF-I and basal GH < 1.0 ng/mL.
Results: Twenty-eight somatotropinomas were studied. Eleven patients (39.3%) were controlled with SA therapy. Mean Ki-67 LI was higher in non controlled patients than in those controlled with SA (3.77 and 0.39 respectively, p=0.007). SSTR2 was correlated with control as well (p=0.04). There was no difference between Ki-67 in patients with high or low SSTR2 expression (2.03 and 2.75, respectively, p=0.651). After multivariate analysis, Ki-67 (p<0.001) and SSTR2 (p=0.02) remained with statistical significance.
Conclusions: Ki-67 is a predictor of SA response in acromegaly independently of the SSTR2 expression.

(1) Fusco A et al., J Clin Endocrinol Metab 2008;93:2746-50

(2) de Pinho LKJ et al. Neuroendocrinology 2010; Dec 18[Epub ahead of print]

Nothing to Disclose: LKJdP, LEAW, LVN, CMT, MRG

Pub # P3-289

Session Information POSTER SESSION: CLINICAL - Diverse Issues in Acromegaly (1:30 PM-3:30 PM)

Title Pharmacodynamics and Safety of an 84-mg Subcutaneous Octreotide Hydrogel Implant for the Treatment of Acromegaly

Author String C Chieffo, M Ryan, C Malott, LA Frohman
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Body **Background:** Octreotide, the first somatostatin analog (SSA), requires monthly injections in its approved long-acting form. We present efficacy and safety data from a randomized, open-label, phase II study evaluating nonhydrated (NHD) and hydrated (HD) 84-mg octreotide hydrogel implants that provide controlled drug release for 6 months.

Methods: Adults with a growth hormone [GH]-secreting tumor and complete (GH <1.0 ng/mL after oral glucose tolerance test and insulin-like growth factor 1 [IGF-1] normalization) or partial ([ge]30% decrease in GH/IGF-1 vs pretreatment) octreotide response had a NHD or HD implant inserted subcutaneously in their upper arm. Implants were removed after 6 months. Blood was drawn at screening, baseline (preinsertion), and weeks 1-6, 8, 12, 16, 20, and 24 for analysis of GH and IGF-1.

Results: 34 patients (n=17 each group) enrolled/finished the study (safety population); 1 patient was excluded from the efficacy analysis because the HD implant was damaged at insertion. The population was heterogeneous; all patients had previously been treated with SSAs, but not all were currently on treatment. Mean \pm SD IGF-1 levels in NHD patients were: baseline, 369 \pm 172 ng/mL; week 2, 258 \pm 129 ng/mL; week 12, 281 \pm 151 ng/mL; week 24, 310 \pm 147 ng/mL. Mean \pm SD IGF-1 levels in HD patients were: baseline, 482 \pm 244 ng/mL; week 2, 288 \pm 128 ng/mL; week 12, 386 \pm 176 ng/mL; week 24, 386 \pm 195 ng/mL. Mean \pm SD GH levels in NHD patients were: baseline, 10.0 \pm 11.3 ng/mL; week 2, 2.9 \pm 2.2 ng/mL; week 12, 4.9 \pm 5.2 ng/mL; week 24, 6.3 \pm 5.4 ng/mL. Mean \pm SD GH levels in HD patients were: baseline, 22.0 \pm 40.3 ng/mL; week 2, 4.8 \pm 5.5 ng/mL; week 12, 5.6 \pm 6.9 ng/mL; week 24, 5.4 \pm 7.9 ng/mL. In the NHD and HD groups, 53% and 50% of patients had mean on-treatment normalized IGF-1 and 35% and 44% had GH <2.5 ng/mL, respectively. There were no serious adverse events (AEs). AEs possibly related to treatment reported in [ge]2 patients in both groups were diarrhea (11.8% NHD, 23.5% HD) and cholelithiasis (11.8% each group). Three patients had new incidence of gallstones (n=2, NHD; n=1, HD). Rash, inflammation or irritation at the implantation site occurred in 3 patients (n=1, NHD; n=2, HD). One patient, whose HD implant was damaged at insertion, had no treatment-related AEs.

Conclusions: NHD and HD octreotide hydrogel implants provided comparable stable suppression of IGF-1 and GH over 6 months and were well tolerated. Phase III studies of the 84-mg NHD octreotide hydrogel implant are ongoing.

Sources of Research Support: Endo Pharmaceuticals Inc.

Disclosures: CC: Employee, Endo Pharmaceutical. MR: Employee, Endo Pharmaceuticals Inc. CM: Employee, Endo Pharmaceuticals Inc. LAF: Consultant, Endo Pharmaceuticals Inc.

Pub # P3-290

Session Information POSTER SESSION: CLINICAL - Diverse Issues in Acromegaly (1:30 PM-3:30 PM)

Title Pharmacokinetics of an 84-mg Subcutaneous Octreotide Hydrogel Implant for the Treatment of Acromegaly

Author String C Chieffo, SA Bai, H Quandt, C Malott
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Body **Background:** Somatostatin analogs (SSAs), the typical pharmacotherapy for acromegaly, require at least monthly injections. To reduce the administration frequency for octreotide, the first available SSA, a subcutaneous octreotide hydrogel implant has been developed to continuously deliver a daily therapeutic dose of octreotide for 6 months. A phase II open-label study was conducted to evaluate the pharmacokinetic (PK) profile of hydrated (H, placed in saline for 3-7 days before implantation) and nonhydrated (NH) 84-mg octreotide hydrogel implants. Insertion of a NH implant would increase the convenience of this dosage form. **Methods:** Patients aged [ge]18 years with a growth hormone-secreting tumor and complete/partial response to octreotide were randomized 1:1 to have a H or NH 84-mg octreotide implant inserted subcutaneously in the upper arm. Blood samples for PK analysis were collected at screening, day 1 (baseline [before insertion] and 6, 12, 24, and 36 h after insertion), days 3-14, and weeks 3-6, 8, 12, 16, 20, and 24; implants were removed after 6 months. Octreotide levels were measured using HPLC/MS/MS, and maximum serum concentration (C_{max}), time to C_{max} (t_{max}), and area under the drug concentration-time curve (time 0 to the last measurable concentration, AUC_{0-t}) were derived. **Results:** 33 patients (H, n=16; NH, n=17) were included in the PK analysis. Mean±SD C_{max} of the implants was 1291±628 (NH) and 2399±883 (H) pg/mL and AUC_{0-t} was 4182±1239 (NH) and 5922±1708 (H) pg [times]mo/mL. Median t_{max} occurred ~1 week later in the NH group (480 h [3 wk] vs 300 h [2 wk] for the H implant; $P=0.001$, Wilcoxon rank sum test). Mean octreotide concentrations reached a plateau at week 6 for the NH implant (796±195 pg/mL) and week 8 for the H implant (1006±368 pg/mL) and remained relatively constant throughout the rest of the treatment period (NH: wk 8, 762±260; wk 12, 616±200; wk 16, 665±187; wk 20, 612±167; wk 24, 528±162 pg/mL; H: wk 12, 836±261; wk 16, 823±346; wk 20, 716±220; wk 24, 623±193 pg/mL). The difference in octreotide concentrations between the NH and H implants was most evident during the first 6 weeks after implantation. Thereafter, differences were generally small. **Conclusions:** NH and H octreotide hydrogel implants provided constant octreotide release over 6 months and showed similar PK profiles during most of the treatment period. Because of the convenience of using a NH implant, the 84-mg NH implant is currently being tested in phase III studies.

Sources of Research Support: Endo Pharmaceuticals Inc.

Disclosures: CC: Employee, Endo Pharmaceutical. SAB: Employee, Endo Pharmaceuticals Inc. HQ: Employee, Endo Pharmaceuticals Inc. CM: Employee, Endo Pharmaceuticals Inc.

Pub # P3-291

Session Information POSTER SESSION: CLINICAL - Diverse Issues in Acromegaly (1:30 PM-3:30 PM)

Title Rates of Biochemical Response in Patients with Acromegaly Treated with a Subcutaneous Octreotide Hydrogel Implant

Author String C Chieffo, M Ryan, C Malott, MR Gadelha, LA Frohman
Endo Pharmaceuticals Inc, Chadds Ford, PA; Endo Pharmaceuticals Inc, Chadds Ford, PA; Endo Pharmaceuticals Inc, Chadds Ford, PA; Federal University of Rio de Janeiro, Rio de Janeiro, Brazil; University of Illinois at Chicago, Section of Endocrinology, Diabetes, & Metabolism, Chicago, IL

Body **Objective:** To assess the rates of biochemical response in patients with acromegaly treated with an octreotide hydrogel implant (OHI).
Methods: In 2 phase II open-label studies, patients were randomized 1:1 to receive 1 (n=5) or 2 (n=6) 52-mg OHI in study 1 or a hydrated (n=16) or nonhydrated (n=17) 84-mg OHI in study 2. Patients [ge]18 years of age with confirmed octreotide response ([ge]30% decrease in insulin-like growth factor 1 [IGF-1] and growth hormone [GH] vs pretreatment) had an implant inserted subcutaneously into their upper arm. The implant was removed after 6 months. Serum GH and IGF-1 were determined pretreatment (screening and just before insertion) through month 6. Mean on-treatment GH and IGF-1 levels were categorized with reference to pretreatment GH level (<5 or [ge]5 ng/mL). Results were compared with the octreotide long-acting release (OLAR) and lanreotide injection prescribing information (PI).
Results: The overall response rates during OHI treatment in the 52- and 84-mg studies, respectively, showed mean GH <5 ng/mL in 91% (10/11) and 70% (23/33) of patients, <2.5 ng/mL in 73% (8/11) and 39% (13/33) and <1 ng/mL in 27% (3/11) and 6% (2/33); IGF-1 normalized in 27% (3/11) and 52% (17/33) of patients. Categorizing patients based on pretreatment GH levels, 3 (52-mg study) and 14 (84-mg study) patients had pretreatment GH <5 ng/mL. During OHI therapy, all 17 (100%) achieved mean GH <5 ng/mL, 100% (3/3) and 57% (8/14) achieved GH <2.5 ng/mL, 67% (2/3) and 14% (2/14) achieved GH <1 ng/mL, and 67% (2/3) and 79% (11/14) normalized IGF-1. Of those patients with pretreatment GH [ge]5 ng/mL in the 52- (n=8) and 84-mg (n=19) studies, respectively, during OHI therapy, 88% (7/8) and 47% (9/19) had GH <5 ng/mL, 63% (5/8) and 26% (5/19) had GH <2.5 ng/mL, 13% (1/8) and none (0/19) had GH <1.0 ng/mL, and 13% (1/8) and 32% (6/19) normalized IGF-1. In comparison, hormone response data from the OLAR and lanreotide PI show comparable rates of GH suppression and IGF-1 normalization.
Conclusions: In patients with acromegaly, the 52- and 84-mg octreotide hydrogel implants provided acceptable suppression of GH and IGF-1 over 6 months, with similar response rates to those previously reported for other somatostatin analogs.

Sources of Research Support: Endo Pharmaceuticals Inc.

Disclosures: CC: Employee, Endo Pharmaceutical. MR: Employee, Endo Pharmaceuticals Inc. CM: Employee, Endo Pharmaceuticals Inc. LAF: Consultant, Endo Pharmaceuticals Inc. Nothing to Disclose: MRG

Pub # P3-292

Session Information POSTER SESSION: CLINICAL - Diverse Issues in Acromegaly (1:30 PM-3:30 PM)

Title Tumor Shrinkage and Improved Headache with Octreotide in a TSH and GH Co-Secreting Pituitary Macroadenoma

Author String AS Cheung, NF Maartens, S Furlanos
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Body TSH secreting tumours (TSHoma) constitute 0.5% of all pituitary tumours. Of these, 30% co-secrete GH, prolactin or gonadotropins in order of decreasing prevalence (1). In GH/TSH co-secreting tumours, thyrotoxicosis may be milder than expected and acromegalic features (present in 95%) may overshadow hyperthyroid features.

A 53-year-old previously well woman presented with 2 years of progressively worsening headaches. She had associated fatigue, postural dizziness and nausea. Four years prior, she had lost 25 kg in weight (body mass index 21) and her shoe size had increased by 1 size.

Examination features were consistent with acromegaly and there were signs of thyrotoxicosis with tachycardia and fine tremor of her hands. MRI pituitary confirmed a 2.2x2.5x1.8cm pituitary macroadenoma abutting the optic chiasm. Her growth hormone(GH) was 34.2mIU/L(<24) and IGF-1 67.3nmol/L(11.3-30.9). Thyroid function tests revealed TSH 1.16mIU/L(0.1-4.0) with elevated FT4 64.4pmol/L(9.0-26.0)and FT3 15.6 pmol/L(3.5-6.5). Alpha subunit was elevated at 0.57IU/L(0.05-0.40). She had evidence of hypopituitarism with oestradiol 32pmol/L, FSH 2.5IU/L, LH 2.1IU/L, cortisol 113nmol/L, ACTH 2.5pmol/L(<20) and a raised prolactin 1019mIU/L(60-620).

Subcutaneous Octreotide therapy (100mcg twice daily) resulted in rapid normalisation of her TSH, FT4 and FT3 in 2 weeks with complete resolution of her headaches. Prolactin fell to 400mIU/L and pituitary MRI demonstrated a 40% reduction in tumour size at 3 months.

The patient was changed to Octreotide-LAR, and had an immediate return in severe headaches necessitating opiate analgesia. With return to short-acting Octreotide, her headaches once again abated. The analgesic effect of short-acting octreotide has also been observed in other patients (2).

Surgery has traditionally been first line treatment, however, achieving euthyroidism preoperatively is paramount. Preoperative octreotide is effective in lowering TSH and GH levels and may have effects in reducing tumour size. With sustained reduction in tumour size, Octreotide may be a suitable first line treatment for patients with TSHoma.

(1) Beck-Peccoz P et al., Endocrine Reviews 1996; 17:610-638
(2) Levy MJ et al., Brain 2005; 128:1921-1930

Nothing to Disclose: ASC, NFM, SF

Pub # P3-293

Session Information POSTER SESSION: CLINICAL - Diverse Issues in Acromegaly (1:30 PM-3:30 PM)

Title Academic vs. Private Study Sites in the Somatuline[reg] Depot (lanreotide) Injection for Acromegaly (SODA Study: A Look at Baseline Patient Characteristics)

Author String R Salvatori, ME Molitch, MB Gordon, S Chang, SM Howell, B Bakker
Johns Hopkins University, Baltimore, MD; Northwestern University Feinberg School of Medicine, Chicago, IL; Allegheny General Hospital, Pittsburgh, PA; Ipsen US, Inc, Brisbane, CA; Ipsen US, Inc, Brisbane, CA

Body **Introduction.** The Somatuline[reg] Depot (lanreotide) Injection for Acromegaly (SODA) study is an ongoing multi-center, observational study of Somatuline Depot (SD)-treated patients with acromegaly, reflecting [ldquo]real world[rdquo] experience with this drug. Both academic (Ac.) and private practice (PP) endocrine centers participate.

Methods. Patients (pts) eligible for enrollment have a clinical diagnosis of acromegaly and are being treated with SD (including the newly prescribed or pts switched to SD from other treatments).

Background. As of December 29, 2010, there were 108 pts enrolled in the SODA database, 64% of pts treated in 18 Ac. sites and 36% are treated in 14 PP sites. A comparison of baseline clinical characteristics between pts enrolled at Ac. vs PP centers is reported here.

Results. Of the 108 pts, 86 (80%) had undergone pituitary surgery (77% Ac. vs 85% PP), 23 (21%) had undergone radiation therapy (22% Ac. vs 21% PP). 44 (41%) were being treated with SSA alone (42% Ac. vs 38% PP), 27 (26%) with SSA and a dopamine agonist (30% Ac. vs 15% PP), and 6 (6%) with SSA, dopamine agonist and pegvisomant (0% Ac. vs 15% PP; p=0.002). 7 received SSA+peg (6%; 7% Ac. vs 5% PP). 24 did not receive long acting SSA prior to enrollment (22%; 20% Ac. vs 26% PP). Most pts' initial SD dose was 90 mg (61% of pts; 62% Ac. vs 59% PP). 21 pts received SD at 60 mg initially (19%; 19% Ac. vs 21% PP). 14 received SD at 120 mg initially (13%; 9% Ac. vs 21% PP). At the time of enrollment, 22 (20%) had a sleep study (22% Ac. vs 18% PP), 44 (41%) had echocardiogram (32% Ac. vs 56% PP; p=0.015), 50 (46%) had colonoscopy (45% Ac. vs 49% PP) and 19 (18%) had gallbladder sonography (14% Ac. vs 23% PP). Thyroid-stimulating hormone (TSH) deficiency was the most common additional pituitary defect reported (35%; 26% Ac. vs 51% PP; p=0.012) followed by hypogonadism (29%; 25% Ac. vs 36 % PP) and hypoadrenalism (13%; 9% Ac. vs 21% PP).

Summary and Conclusions. Baseline differences were observed between Ac. and PP centers; of special interest is the difference in pituitary deficiencies at enrollment in SODA (significant difference in TSH deficiency). Also of interest is the difference in cardiovascular investigations (echocardiograms were performed more frequently in PP).

Sources of Research Support: Scientific Strategy Partners and funded by Ipsen US, Inc.

Disclosures: RS: Researcher, Ipsen; Novartis Pharmaceuticals; Pfizer, Inc.; Advisory Group Member, Novartis Pharmaceuticals. MEM: Researcher, Ipsen; Advisory Group Member, Novartis Pharmaceuticals. MBG: Researcher, Ipsen; Novartis Pharmaceuticals; Pfizer, Inc.; Advisory Group Member, Pfizer, Inc. SC: Employee, Ipsen. SMH: Employee, Ipsen. BB: Employee, Ipsen.

Pub # P3-294

Session Information POSTER SESSION: CLINICAL - Diverse Issues in Acromegaly (1:30 PM-3:30 PM)

Title Long-Term Treatment with Somatostatin Analogs in Acromegaly Increases Life Expectancy. A Comparative Study in Bulgaria and Campania (Italy)

Author String S Vandeva, M Yaneva, RS Auriemma, R Pivonello, E Nachev, K Kalinov, S Zacharieva, A Colao
Medical University - Sofia, Sofia, Bulgaria; University Federico II of Naples, Naples, Italy; New Bulgarian University, Sofia, Bulgaria

Body

Introduction: Acromegaly is a rare disease due to a GH secreting pituitary adenoma in most cases. Undoubtedly, the uncontrolled disorder is associated with several times increased mortality. However, recent studies show normal life expectancy, most probably due to more stringent remission criteria and the application of modern treatment options.

Patients and methods: It was a retrospective cohort study, aiming to compare the impact of different therapeutic modalities, and especially the use of somatostatin analogues (SSAs), on disease control and mortality in acromegaly in two reference centers, Bulgaria and the region of Campania, Italy. Due to fund restrictions in Bulgaria SSAs were available in the routine clinical practice only after 2009. Patients diagnosed from January 1st 1988 till June 1st 2008, were included in the study, respectively 301 vs. 220 for Bulgaria and Campania. Standardized mortality ratio (SMR) was calculated, using data from the statistical institutes of both countries. Patients' characteristics of both cohorts, as well as factors influencing mortality were evaluated by SAS vs 9.2.

Results: Both cohorts had similar baseline biochemical characteristics. In regards to treatment, 88.6% of the Campanian patients have used SSAs vs. none of the Bulgarian patients, who on the other side had more surgery, radiotherapy and dopamine agonists applied. This different approach resulted in significantly better biochemical control in Campania - median final GH (ng/ml) 1.95 (0.8-5.2) vs. 2.85 (1.03-7.95) $p=0.002$; mean final IGF-1 (upper limit of normal) - 1.95 ± 0.73 vs. 1.3 ± 0.97 $p<0.001$. As expected, death cases were significantly more in Bulgaria 35 vs. 7 $p<0.001$ predominantly due to cardiovascular 14 vs. 3 $p=0.045$ and cerebrovascular 13 vs. 0 $p<0.001$ diseases. All-cause mortality of the Bulgarian cohort was significantly greater than the general population SMR 2.19 (1.52-3.04 $p<0.001$). Internal analysis showed influence of GH (>2.5 ng/ml vs. <2.5 ng/ml), IGF-1 and diabetes mellitus on mortality rate ratios: 3.23 (1.41-7.36 $p=0.0037$); 3.96(1.33-11.83 $p=0.0089$); 2.23 (1.01-4.92 $p=0.0484$) respectively. No impact of radiotherapy, hypertension or hypopituitarism could be demonstrated.

Conclusion: The use of SSAs as a modern treatment option results in a better biochemical disease control of acromegaly and thus restores life expectancy to normal. Future studies would further evaluate the impact of other possible factors influencing mortality.

Nothing to Disclose: SV, MY, RSA, RP, EN, KK, SZ, AC

Pub # P3-295

Session Information POSTER SESSION: CLINICAL - Diverse Issues in Acromegaly (1:30 PM-3:30 PM)

Title Use of Pegvisomant in Acromegaly

Author String RW Zilli, MKP Huayllas, LB Tavares, PP Mariani, A Cukiert
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Body

Context: acromegaly is a rare disease and can be cured with surgery in 50% of macroadenomas. Clinic treatment includes the use of drugs such as somatostatin analogs and pegvisomant and can control the disease. Without control, acromegaly has increased mortality rate due cardiovascular disease and it is directly related to the complications of GH excess. Almost 30% do not respond to treatment with somatostatin analogues and radiotherapy takes long time to do effect. Pegvisomant is a genetically engineered, highly selective GHR antagonist that can reduce IGF-1 in 97% of patients.

Objective: to report our experience using Pegvisomant in a referral Center for pituitary tumors

Patients and methods: we evaluated for 16 months prospectively 14 acromegalic patients (7M) with active disease after unsuccessful neurosurgery for invasive macroadenoma and the use of somatostatin analog therapy. Results are expressed in percentage of change in IGF-I levels (basal and maximum decrease).

Results: Age was 45 + 12 years, duration of disease 1-28 y, 57% had radiotherapy, 35% were diabetic, 79% had hypertension. Dose of pegvisomant was 10mg/d SC and 2 patients increased for 20mg/d. Maximum reduction was observed in the group within six months of pegvisomant (66% - p <0,001). An elevation of transaminases was observed in 4 patients. One patient did not normalized IGF-I after 8 months. GH level increased after beginning of pegvisomant (p <0,05) and MRI before and after treatment did not change. (Serum Gh and IGF-1 were measured by Immulite 2000, DPC Los Angeles, CA). Diabetic patients had better control and decreased the dose of daily insulin. No lipodistrophy was observed.

Conclusion: our results show that pegvisomant was safe in acromegalic patients and the compliance was 100%. No serious adverse effects were observed and no changes in MRI were reported.

Nothing to Disclose: RWZ, MKPH, LBT, PPM, AC

Pub # P3-296
Session Information POSTER SESSION: CLINICAL - Diverse Issues in Acromegaly (1:30 PM-3:30 PM)
Title Pegvisomant (Somavert^[reg]): Long-Term Treatment Outcomes of Acromegaly in Everyday Clinical Practice -- Cross-Sectional Observations from ACROSTUDY
Author String M Buchfelder, BMK Biller, J Cara, E Ghigo, J Hey-Hadavi, M Koltowska-Haggstrom, N Rajjicic, AJ van der Lely, F Lundgren, B Saller, J Sanocki, CJ Strasburger, SM Webb, T Brue
University of Erlangen-Nuremberg, Erlangen, Germany; Massachusetts General Hospital, Boston, MA; Pfizer Inc, New York, NY; Universit[grave] di Torino, Turin, Italy; Pfizer Health AB, Sollentuna, Sweden; Erasmus University Medical Centre, Rotterdam, Netherlands; Pfizer Ltd, Tadworth, UK; Pfizer Inc, New London, UK; Charit[umlaut]tsmedizin, Berlin, Germany; Universitat Aut[ograve]noma de Barcelona, Barcelona, Spain; Universit[grave] de la M[editerran]e, Marseille, France

Body
Introduction: Somavert^[reg] is a growth hormone receptor blocker, which inhibits hepatic production of insulin-like growth factor I (IGF-I).
Objective: To evaluate safety [liver function tests (LFT), pituitary tumour changes and insulin-like growth factor I (IGF-I)] during Somavert^[reg] treatment of subjects with acromegaly in everyday clinical practice.
Methods/design: ACROSTUDY is an open-label, international, prospective, non-interventional, post-marketing surveillance study monitoring the long-term safety of Somavert.
Results: As of December 31, 2009 data from 1288 (51% men) patients were available. Mean (SD) age (yrs) of patients at diagnosis of acromegaly was 42 (13.5) and at Somavert start - 50 (14.0). Mean (SD) duration of Somavert treatment was 3.7 (1.91) yrs.
In subjects with normal liver function tests at Somavert start (n=536), <1% of subjects had a laboratory report of an ALT or AST elevation of >3x upper limit of normal (ULN) at any time point during their follow up in ACROSTUDY.
Out of 936 patients who had pituitary imaging results reported, there was no change in tumor volume noted in 78.8%, while in 12.8% a decrease and in 7.2% an increase were reported. In 1.5%, both an increase and a decrease were reported. Central reading of the MRIs was performed in 45/67 subjects with reported increase and tumor growth was confirmed in only 14. Overall, central MRI reading assessed tumor change as increase in a total of 30 subjects.
At Somavert^[reg] start (n=839) 86% of patients were reported as having increased IGF-I concentrations. After 1 year of treatment (n=769) 57% of subjects had normal IGF-I concentration (mean dose 14.8mg/day). Subsequent IGF-I normalization rates were reported as follows: 2yrs (n=705), mean dose 15.6mg/day - 60%; 3yrs (n=563), mean dose 15.9mg/day - 60%; 4yrs (n=406), mean dose 16.8mg/day - 63%; 5yrs (n=253), mean dose 17.5mg/day - 63%.
Conclusion: The ACROSTUDY safety data from a large cohort of subjects treated with Somavert for acromegaly indicates a satisfactory safety profile in clinical practice, especially concerning liver function tests and pituitary tumor growth. Although ACROSTUDY is not an efficacy study, around two-thirds of patients experienced normalization of IGF-I on relatively low doses of Somavert without any indication of reduction in IGF-I normalization during long-term treatment.

Sources of Research Support: Pfizer Inc.

Disclosures: BMKB: Principal Investigator, Novartis Pharmaceuticals; Pfizer, Inc.; Consultant, Novartis Pharmaceuticals; Pfizer, Inc. JC: Employee, Pfizer, Inc. H-H: Employee, Pfizer, Inc. MK-H: Employee, Pfizer, Inc. NR: Employee, Pfizer, Inc. FL: Employee, Pfizer, Inc. BS: Employee, Pfizer, Inc. JS: Employee, Pfizer, Inc. CJS: Speaker, Novartis Pharmaceuticals; Pfizer, Inc.; Novo Nordisk; Scientific Board Member, Lilly USA, LLC; Advisory Group Member, Pfizer, Inc.; Clinical Researcher, Ipsen. TB: Advisory Group Member, Ipsen; Novartis Pharmaceuticals; Pfizer, Inc.; Speaker, Ipsen; Lilly USA, LLC; Novartis Pharmaceuticals; Novo Nordisk; Pfizer, Inc.; Clinical Researcher, Ipsen; Novartis Pharmaceuticals; Pfizer, Inc. Nothing to Disclose: MB, EG, AJvdL, SMW

Pub # P3-297

Session Information POSTER SESSION: CLINICAL - Diverse Issues in Acromegaly (1:30 PM-3:30 PM)

Title Clinical, Biological and Genetic Factors Determining the Response to Pegvisomant Therapy in Acromegaly

Author String P Kamenicky, M Rozier, L Maione, S Salenave, C Le Stunf, P Chanson
APHP, Bicetre Hospital, Le Kremlin Bicetre, France; INSERM, Paris, France

Body

Background: The dose of pegvisomant required to normalize serum IGF-I in patients with acromegaly is variable, and depends on disease activity and individual responsiveness to the drug.

Objective: To determine the clinical, biological and genetic factors influencing the therapeutic response to pegvisomant in patients with acromegaly.

Patients and Methods: Retrospective data collection in 53 (23F/30M) patients receiving pegvisomant treatment and followed in Bicetre Hospital from 2003 to 2009. Before pegvisomant therapy, patients had been treated with transphenoidal surgery (n=37), external radiotherapy or gamma-knife (n=11), somatostatin analogues (n=45) and/or dopaminergic agonists (n=21). Median serum IGF-I before pegvisomant treatment was 200% (range, 110-530%) of the age-adjusted upper limit of normal (ULN). Pegvisomant was initiated at the dose of 10 mg/day and was thereafter adjusted by 5-10 mg every 4 weeks until normalization of serum IGF-I.

Fifty patients were genotyped for the full-length (fl) and the exon 3-deleted (d3) growth hormone receptor (GHR) alleles since the d3-GHR genotype was suggested to be associated with a greater response to the drug.

Results: Pegvisomant allowed to obtain a short-term control of acromegaly in 48 (91%) patients. At the end of the follow up (27 months, range 1-77 months), IGF-I was in the normal range in 40 patients (75%). Median serum IGF-I under pegvisomant treatment was 80% ULN (range 35-270% ULN, P<0.0001). Median pegvisomant dose required for acromegaly control was 20mg/day (range 10-80 mg/day) with no significant sex difference. In 18 (34%) patients pegvisomant was used in combination with somatostatin analogues and/or dopaminergic agonists. In a multiple regression model, pegvisomant dose required to normalize serum IGF-I was associated with pretreatment serum IGF-I concentration (P<0.001) and body mass index (BMI, P=0.001), tended to be associated with GHR genotype (P=0.14) and did not correlate with age, gender, previous radiotherapy and baseline GH concentrations.

Conclusion: Pretreatment IGF-I concentration which reflects disease activity and BMI are two major predictive factors of the therapeutic dose of pegvisomant required to obtain control of acromegaly. Genetic variation in the GHR locus seems to play a limited role.

Disclosures: PC: Advisory Group Member, Eli Lilly & Company; Clinical Researcher, Ipsen; Novartis Pharmaceuticals; Novo Nordisk; Pfizer, Inc.; Merck & Co. Nothing to Disclose: PK, MR, LM, SS, CLS

Pub # P3-298

Session Information POSTER SESSION: CLINICAL - Diverse Issues in Acromegaly (1:30 PM-3:30 PM)

Title AcroBel-2: The Belgian Follow-Up Survey on Acromegaly -- Contribution of Pituitary Surgery and Pegvisomant to an Improvement in Disease Control

Author String MA Bex, R Abs, G T'Sjoen, B Corvilain, B Velkeniers, D Maiter
UZ Leuven, Leuven, Belgium; Antwerp, Berchem, Belgium; UZ Gent, Ghent, Belgium; ULB Erasme, Brussels, Belgium; AZ Brussel, Jette, Belgium; UCL St Luc, Brussels, Belgium

Body Two nationwide surveys on acromegaly have been performed in Belgium at an interval of five years, with centralized IGF-I measurements in most patients. The mean IGF-I z-score decreased from 1.87 to 1.19 between the two surveys and a normal IGF-I for age was observed in 56% of 311 vs. 74% of 365 treated patients, in 2004 (AcroBel-1) and 2009 (AcroBel-2) respectively. Factors responsible for this improvement were investigated. A selection bias was unlikely as the follow-up rate was higher in patients with previously active (83%) or medically controlled disease (71%) compared to those with surgical remission (61%). The proportion of patients with microadenoma did not increase. * In 225 patients with sampling in both surveys, a normal IGF-I was observed more frequently in AcroBel-2 (80%; mean IGF-I z-score 1.02) than in AcroBel-1 (52%; mean z-score 2.10), due to a first or additional surgery (n=20, 10% microadenoma; overall remission in 45%), long-term effects of previous radiotherapy (n=84) and initiation or intensification of medical therapy, including pegvisomant (n=21; control in 67%). * In 106 patients newly diagnosed and treated since the first survey, 63 % had a normal IGF-I (mean z-score of 1.58). Seventy-five % had pituitary surgery (11% microadenoma) and the overall remission rate (including low or suppressible GH) was 43%, but reached 53% and 68% in two centers responsible for over half of surgeries. Surgical procedures elsewhere (38 patients in 18 centers) allowed remission in 23% (range 0-100%). Radiotherapy was used in 3 cases only. Primary medical therapy (mostly somatostatin analogues (SSA), n=24) normalized IGF-I in 42%; adjuvant medical therapy with SSA or dopamine agonists (n=24) in 50%, and combined treatment with SSA and pegvisomant following surgery (n=11) in 64%. * The remaining 34 patients from AcroBel-2 were diagnosed before 2003 but not included in the first survey (33 operated; 13 irradiated; 10 on medical therapy). A normal IGF-I was present in 74 % and their mean IGF-I z-score was 1.05.

Conclusion: In this recent Belgian acromegaly survey, a more frequent disease remission or control was observed, due to a greater use of surgery with improved outcome and to intensification of medical treatment including the introduction of pegvisomant.

Sources of Research Support: Novartis Pharmaceuticals Belgium; Clinical Doctoral Grant of the Research Foundation - Flanders (FWO) awarded to MB.

Disclosures: MAB: Research Funding, Novartis Pharmaceuticals; Principal Investigator, Novartis Pharmaceuticals; Clinician, Pfizer, Inc.; Novartis Pharmaceuticals; Investigator, Ipsen. RA: Speaker, Pfizer, Inc.; Advisory Group Member, Pfizer, Inc.; Novartis Pharmaceuticals; Ipsen; Clinician, Pfizer, Inc.; Novartis Pharmaceuticals; Research Funding, Novartis Pharmaceuticals. GT: Clinician, Lilly USA, LLC; Novartis Pharmaceuticals; Ipsen; Investigator, Bayer, Inc.; Coinvestigator, Novartis Pharmaceuticals. BC: Investigator, Novartis Pharmaceuticals; Clinician, Novartis Pharmaceuticals; Medical Advisory Board Member, Novartis Pharmaceuticals. BV: Speaker, Pfizer, Inc.; Coinvestigator, Novartis Pharmaceuticals; Clinician, Novartis Pharmaceuticals; Pfizer, Inc.; Medical Advisory Board Member, Novartis Pharmaceuticals; Ipsen; Pfizer, Inc. DM: Clinician, Ipsen; Novo Nordisk; Pfizer, Inc.; Novartis Pharmaceuticals; Investigator, Novartis Pharmaceuticals.

Pub # P3-299

Session Information POSTER SESSION: CLINICAL - Diverse Issues in Acromegaly (1:30 PM-3:30 PM)

Title A 20-Year Single Canadian Center Follow-Up Highlights the Need for Multimodal Therapy in the Majority of Acromegalic Patients

Author String R Alagha, C Murray, L Kindon, S Ezzat
University Health Network, Toronto, Canada

Body
Background: Acromegaly is a disease characterized by growth hormone (GH) hypersecretion resulting in high circulating hormone levels. Normalization of the GH-target insulin-like growth factor-1 (IGF-1) is considered essential in managing associated risks including heart, lung, joint, and metabolic complications.
Aim: To determine the therapies required to achieve long-term IGF-1 normalization in acromegalic patients managed by a single centre in a publically-funded health care system.
Methods: A retrospective review of 113 acromegalic patients attending our tertiary care centre over the last 20 years was performed. The most recent IGF-1 values were adjusted for age and gender.
Results: Normalization of IGF-1 was ultimately achieved in 86 (76%) of patients. All but 6 underwent trans-sphenoidal surgery (TSS). Of those who achieved normalization only a minority (n=16; 19%) did not require additional therapies. Thirty patients (35%) achieved remission with post-operative octreotide alone. Another 8 patients (9%) required post-operative octreotide and pegvisomant. Another 5 patients (6%) required two operations, octreotide, and pegvisomant. Another 7 patients (8%) achieved disease control with post-operative octreotide and radiotherapy. A repeat operation alone achieved normalization in 3 (3%) patients. Three patients (3%) were controlled with octreotide alone. Three patients (3%) reached remission with 2 TSS, octreotide, and radiation. Three patients (3%) were controlled with 2 TSS, octreotide, pegvisomant, and radiotherapy. Two patients (2%) achieved remission with TSS and radiotherapy. Two patients (2%) were controlled with octreotide and pegvisomant. Two patients (2%) achieved remission with TSS, radiotherapy, octreotide and pegvisomant. One patient (1%) was controlled with 2 TSS and radiotherapy. One patient (1%) achieved remission with 2 TSS and octreotide. Despite these combination therapies, 27 patients (24%) of the entire cohort could not sustain long-term IGF-1 normalization.
Conclusion: Long-term follow-up demonstrates the need for multiple modalities in managing the majority of acromegalic patients.

Nothing to Disclose: RA, CM, LK, SE

Pub # P3-300

Session Information POSTER SESSION: CLINICAL - Diverse Issues in Acromegaly (1:30 PM-3:30 PM)

Title Lost to Follow-Up in Acromegaly, Results of ACROSPECT Observational Study

Author String B Delemer, C Reines, T Brue, C Cortet Rudelli, I Raingeard, Y Reznik, X Parot, O Chabre
CHU de Reims, Reims, France; Novartis, Rueil Malmaison, France; CHU la Timone, Marseille, France;
Clinique Marc Linquette, Lille, France; Hopital Lapeyronie, Montpellier, France; Hopital Cote de Nacre,
Caen, France; Hopital Albert Michallon, La Tronche, France

Body Lost to follow-up (LTFU) is a frequent problem in chronic diseases not evaluated yet in acromegaly despite the long-term need of medical treatment and complications survey in this disease.

Objectives
Evaluation of the prevalence of LTFU patients in a multi-center cohort, characteristics of defaulters and description of their evolution after they have been searched and found.

Méthods
Observational multicentric (25 centers study). Each center was asked for the number of acromegalic patients followed up between 1997 and 2007 and for the number of the LTFU patients in the same period. Patients LTFU were defined as the ones who missed follow-up appointments in at least 2 years. These patients and their GP were recalled by phone and mail and asked about reasons to non-attendance. They were invited either to come to the former center for evaluation of acromegaly or to fill up a medical questionnaire.

Results
482 patients under 2252 were identified as LTFU, mean 21.4% of the cohort with a variation between the centers from 7.7 to 49%. At the last evaluation before LTFU, IGF1 was normal without treatment in 54%, normal with a medical treatment in 16% and not controlled in 30% of the patients. Recent news have been collected in 362 patients with 62 dead. Most frequent reason of non attendance was follow-up by another physician in 69%. Percentage of patients with medical treatment decreased from 28% to 15%. Among the 87 patients who came back in the center, acromegaly was not controlled in 20% and the treatment had to be modified in 17%, lack of medical survey was observed in 16%.

Conclusion
In acromegaly LTFU concerns 21.4% of the patients. Treatments are stopped in these patients in half of the cases. When the patients are reevaluated 20% are not controlled. As acromegaly can now be controlled quality of follow-up is a real clinical challenge.

Sources of Research Support: Novartis Pharmaceuticals.

Disclosures: CR: Employee, Novartis Pharmaceuticals. XP: Employee, Novartis Pharmaceuticals. Nothing to Disclose: BD, TB, CCR, IR, YR, OC

Pub # P3-301
Session Information POSTER SESSION: CLINICAL - Diverse Issues in Acromegaly (1:30 PM-3:30 PM)
Title Octreotide and Dopamine Agonist Treatment of Acromegaly Resulting in Apparent Remission of Chronic Lymphocytic Leukemia
Author String J Del Rivero, A Sacerdote, G Bahtiyar
Woodhull Medical Center, Brooklyn, NY; SUNY Downstate Medical Center, Brooklyn, NY; NYU School of Medicine, New York, NY

Body **Background:** Somatostatin receptors are overexpressed in many tumors of various origins. Somatostatin receptors are expressed in circulating lymphoid cells and there is evidence that somatostatin can modulate lymphocytic functions via its receptors and is likely to play an inhibitory role during the development of lymphoproliferative disease. There is increasing interest in using Octreotide also known as SMS 201-995 (SMS) in cancer treatment due its potentially antiproliferative effect and apoptotic actions.
Clinical case: A 53 year-old male was diagnosed with a pituitary adenoma in 1988. He was started on bromocriptine with reported fair response. In 1994 he continued on bromocriptine until 1998 when he noticed an increase in shoe and nose size. His laboratory findings were consistent with acromegaly: growth hormone by immunoassay of 5.9 ng/ml (≤ 10), IGF-1 by electrochemiluminescence=589 ng/dl (46-284), IGF binding protein-1 by RIA= 4.3 ng/ml (10-40), growth hormone antibodies by RBA were negative. In 2002 he was further evaluated due to persistently elevated white blood cells (WBC) of 14,100/uL and elevated lymphocytes of 56%, peripheral blood smear showed lymphocytosis with smudge cells; he was diagnosed with B-cell chronic lymphocytic leukemia based on flow cytometry. He was started on octreotide LAR, initially at 10 mg, later titrated to 20 mg and bromocriptine was changed to an equivalent twice weekly dose of cabergoline. Pioglitazone 15 mg daily was added to treat IFG. Over 6 years we observed a WBC decrease to 8,900/uL and lymphocytes to 48%, IGF-1 to 181 ng/dl and growth hormone to 2.3 ng/ml respectively. His pituitary tumor is no longer detectable.
Conclusion: SMS has been clinically useful in oncology, endocrinology and hematology for its strong inhibitory activity on multiple cell type proliferation and hormonal secretory processes. The presence of somatostatin receptors on immune cells indicates that SMS may be one of the regulators of immune cell function in humans. Further, prolactin is an autocrine factor for a human leukemic cell line. Insulin has proliferative effects via binding to IGF-1 receptors and by reduction of IGF-binding protein. In addition, human B lymphocytes express PPAR- γ and PPAR- γ agonists are reported to be lethal to B lymphocytes; B lymphocytes also express dopamine receptors and dopamine is reported to impair their mitochondrial membrane potential. Thus, the therapeutic effect on the patient's CLL is multifaceted.

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Nothing to Disclose: JDR, AS, GB

Pub # P3-302

Session Information POSTER SESSION: CLINICAL - Diverse Issues in Acromegaly (1:30 PM-3:30 PM)

Title Functional and Structural Carotid Wall Changes in Acromegaly Not Reversible after 15 Months of Disease Control

Author String S Galoiu, R Jurcut, A Vladaia, A Florian, M Purice, C Ginghina, M Coculescu
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Body Long term GH and IGF1 excess in acromegaly (ACM) and the high prevalence of cardiovascular risk factors (CVF) lead to vascular changes. Current therapies can normalize GH and IGF1 levels and restore the mortality rate to that of general population.
Aims: to establish if vascular changes in ACM are reversible after disease control.
Materials and methods: 62 consecutive patients with ACM (45.7±11.8yrs, 26M/ 36F), were investigated by classical CVF, random serum GH and during OGTT (sensitivity 0.04ng/ml) and IGF1 levels, right common carotid (RCC) ultrasonography. The structural (carotid diameter, intima-media thickness index - IMT) and functional parameters (augmentation index AIX, elastic modulus Ep and local pulse wave velocity-PWV) with e-tracking were measured and compared to 21 age and sex matched non-acromegalic controls (group C) (40.7±11.8yrs, 10M/11F).
Results: Patients with ACM were divided into 2 groups, using current criteria of active ACM (A; n=53) and controlled ACM (B; n=9). The estimated disease duration was 10.8±7.3yrs in A group and 14.6±12.6yrs in B group (p=NS). CVF's prevalence was no statistically different between A and B group. The disease free interval in B group was 1.3±0.7 yrs.
All the patients with ACM had higher RCC diameters (6.4±0.6mm vs. 5.7±0.6mm, p<0.001) and higher IMT vs. group C (0.72±0.13mm vs. 0.58±0.08mm, p<0.001); p=NS A vs. B. IMT was correlated with age (r=0.3, p=0.008), estimated disease duration (r=0.4, p=0.004) and systolic blood pressure (r=0.5, p<0.001). At multiple regression analysis, the predictors of IMT were disease duration and systolic blood pressure (r=0.6, p=0.005).
Group A patients had increased functional parameters of carotid wall versus controls: Aix- 27.2±32.6% in A group vs. 5.8±10.6% in C group, p=0.001, but non statistically different vs. B group- 25.9±19.1%, p=0.04 B vs. C; PWV was 6.3±1.6m/s in group A vs. 5.8±1.3m/s in group B vs. 5.4±0.7m/s in group C, p=0.009 A vs.C. There was a tendency (nonstatistically proved) for decreasing of all indices of arterial stiffness in patients with controlled GH versus patients with active ACM.
Conclusions: patients with both active and controlled ACM showed structural changes, dependent on disease duration and systolic blood pressure. There was a tendency for functional, but not structural parameters to decrease in ACM after 1.3 years of disease control. It is tempting to suggest that a longer disease free interval should normalize the functional parameters.

Nothing to Disclose: SG, RJ, AV, AF, MP, CG, MC

Pub # P3-303

Session Information POSTER SESSION: CLINICAL - Diverse Issues in Acromegaly (1:30 PM-3:30 PM)

Title GH Replacement Improves Quality of Life and Metabolic Parameters in Cured Acromegalic Patients with Growth Hormone Deficiency

Author String C Giavoli, E Verrua, E Ferrante, CL Ronchi, E Profka, S Bergamaschi, E Sala, A Lania, M Arosio, A Spada, P Beck-Peccoz

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Body Acromegalics frequently develop GH deficiency (GHD) after surgery and/or radiotherapy for the primary pathology. Effects of GH replacement in such a patients have been so far poorly studied. Aim the present open-labeled, prospective study was to evaluate whether metabolic parameters and quality of life are improved by GH replacement in cured acromegalic patients with GHD. The whole cohort included 40 GHD subjects [mean age (SD): 48±10, BMI 27±3 kg/m²]: 8 were acromegalics treated with rhGH (Group A), whereas 12 age- and sex-matched acromegalics who refused treatment (Group B) and 20 GHD subjects with previous non-functioning pituitary adenoma on rhGH (Group C) served as controls. IGF-I levels, lipid profile, glucose metabolism (fasting and post-OGTT glucose, insulin resistance, HbA1c), anthropometric parameters [BMI, waist circumference, body fat percentage (BF%)] and quality of life [evaluated by the Quality of Life Adult GHD Assessment (QoL-AGHDA)], were evaluated in all patients at the time of GHD diagnosis and after 12 months of rhGH in Group A and C (mean dose 0.28 ±0.02 mg/day) or followed-up in Group B. At baseline, Group B showed higher IGF-I levels evaluated as standard deviation score than Group A and C (-1.4±0.8 vs -2.2±0.5 and -2.4±0.5, respectively, *P*=0.01), better QoL-AGHDA scale Z-scores (-0.4±0.1 vs -1.9±0.9, *P*=0.01) and higher post-OGTT glucose levels (127±34 vs 90±32 mg/dl, *P*=0.05) than Group A. No difference among Groups was recorded for other parameters. After 12 months, IGF-I levels significantly increase in group A and C (-0.6±1.3 and -0.7±0.9 SDS, respectively, *P*<0.004 vs baseline). A decrease in BF% (from 36.3±5.2 to 31.7±2.4 and from 33±9 to 30±9 in Group A and C, respectively, *P*<0.01) and total-cholesterol (from 252±50 to 203±57 and from 261±56 to 209±51 mg/dl in Group A and C, respectively, *P*=0.04) was observed. QoL-AGHDA scale scores significantly improved on rhGH (from -1.9±0.9 to -0.9±1.2 and from -1.5±1.1 to -0.7±1.4, in Group A and C respectively, *P*<0.01). In Group A, fasting and post-OGTT glucose levels, HbA1c and HOMA did not change on rhGH. No side effects were recorded. In conclusion, in GHD acromegalics GH therapy improved body composition, lipid profile and quality of life as in patients with GHD and previous non-functioning pituitary adenoma, without deterioration of insulin resistance. GH replacement therapy should therefore be considered in these patients, as in patients with GHD from other causes.

Nothing to Disclose: CG, EV, EF, CLR, EP, SB, ES, AL, MA, AS, PB-P

Pub # P3-304

Session Information POSTER SESSION: CLINICAL - Prolactinomas, Thyrotropinomas (1:30 PM-3:30 PM)

Title Prolactin Secretion in Healthy Adults Is Determined by Gender, Age and Body Mass Index

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Body Recent reports on the regulation of the somatotrophic, corticotrophic and TSH axis have shown that hormone secretion in healthy adults is influenced by gender, age and BMI, although to various extents for each system (1-3). No such data is yet available for prolactin. We therefore analyzed the 24 h serum PRL concentration series, by blood sampling 74 healthy adult volunteers (41 f, and 33 m) at 10 min intervals. The age range was 21-77 yr, and BMI range 18-39 kg/m². Data were analyzed with an operator-independent Matlab-based deconvolution program and approximate entropy program. In the multivariable regression analysis with gender, age and BMI as independent variables mean 24h PRL concentration and minimum PRL concentration were sex-dependent (P<0.001 and 0.017, respectively), while the maximal 24h PRL concentration was determined by gender (P<0.001) and negatively by age (P=0.003). Basal (non-pulsatile) secretion depended on BMI (P=0.039) and pulsatile secretion on gender (P=0.003). Mean pulse mass was determined by gender (P=0.001) and negatively by age (P=0.038). PRL secretion parameters with the exception of basal secretion (mean, pulsatile, mean mass per burst and total 24h secretion) were larger in women than in men (P=0.001 or less). Postmenopausal women secreted less PRL than premenopausal females by diminished basal and pulsatile components (P=0.01 and 0.003, respectively). In contrast to other hormone systems, approximate entropy was not gender, age or BMI-dependent. This study shows that experiments in the human involving PRL should be controlled for gender, age and BMI.

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(2)Veldhuis JD et al J Clin Endocrinol and Metab 2009;94:4045-52
(3)Veldhuis JD et al J Clin Endocrinol and Metab 2011;96:115-21

Nothing to Disclose: FR, HP, DMK, JDV

Pub # P3-305

Session Information POSTER SESSION: CLINICAL - Prolactinomas, Thyrotropinomas (1:30 PM-3:30 PM)

Title Changes in Serum Prolactin (PRL) Concentrations in Subjects with Macroprolactinemia Having Normal Free PRL Levels during Two Years' Follow-Up

Author String N Hattori, T Ishihara, A Shimatsu
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Body Macroprolactinaemia is characterized by a predominant macroprolactin molecule in the serum and frequently causes hyperprolactinaemia. The prevalence is between 15-25% in patients with hyperprolactinaemia and 3.68% in normal subjects. We recently reported that macroprolactinaemia is a relatively stable condition in patients with hyperprolactinaemia. Macroprolactinaemic patients, who were found among hyperprolactinaemic patients, often had concurrent causes of hyperprolactinaemia. Therefore it is difficult to analyze the long-term changes in serum PRL concentrations due to macroprolactin itself. In this study, we examined the changes in serum PRL concentrations and anti-PRL autoantibody titers in macroprolactinaemic subjects having normal free PRL levels during two years follow-up.

[Subjects and Methods] We examined 35 macroprolactinaemic subjects (25 women and 10 men, aged 43.2 yr) who were found among 1330 normal subjects. Serum PRL concentrations (normal value less than 15 ng/mL), macroprolactin levels determined by polyethylene glycol (PEG) method (PRL precipitation ratio greater than 60% in macroprolactinaemia) and anti-PRL autoantibody titers measured by ¹²⁵I-PRL binding study (¹²⁵I-PRL binding greater than 7.3% in anti-PRL autoantibody-positive sera) were compared in paired serum samples collected in 2 years interval. All macroprolactinaemic subjects had normal monomeric PRL levels in sera.

[Results] Among 12 macroprolactinaemic subjects having hyperprolactinaemia 2 years ago, 10 subjects (83.3%) remained to be hyperprolactinaemic without significant changes in serum PRL levels. One subject showed a decrease in serum PRL concentration from 20 ng/mL to 14.4 ng/mL with a slight decrease in anti-PRL autoantibody titer from 12.0% to 9.9%. The other macroprolactinaemic subject showed a decrease in serum PRL concentration from 25.0 ng/mL to 11.3 ng/mL concurrent with a disappearance of anti-PRL autoantibody (¹²⁵I-PRL binding ratio from 8.7% to 4.7%). Twenty-three subjects with normal serum PRL levels 2 years ago did not show significant changes in serum PRL levels. All 35 macroprolactinaemic subjects except 2 remained to be macroprolactinaemic during 2 years.

[Conclusion] Most macroprolactinaemic subjects remained to be macroprolactinaemic without significant changes in serum PRL concentrations in 2 years interval.

Nothing to Disclose: NH, TI, AS

Pub # P3-306

Session Information POSTER SESSION: CLINICAL - Prolactinomas, Thyrotropinomas (1:30 PM-3:30 PM)

Title Clinical Significance of Anemia Associated with Prolactin-Secreting Pituitary Tumors in Men

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Body An association between prolactin-secreting pituitary adenomas and anemia in male patients have been recently reported. Our aim has been to evaluate the prevalence of anemia in men with prolactinomas and to assess the relationships between hemoglobin concentrations and pituitary function at diagnosis in these patients. In a retrospective analysis 26 male patients with prolactinomas (22 macroprolactinomas and 4 microprolactinomas) were studied. Blood hemoglobin concentration, hematocrit value and baseline hormonal levels were collected at the time of prolactinoma diagnosis. The presence or absence of partial or total hypopituitarism was also evaluated at diagnosis. Logistic regression analysis was used to assess the presence of anemia as a function of serum hormone concentrations and pituitary dysfunction. Patient bearing macroprolactinomas showed significant lower hemoglobin concentrations than those found in patients with microprolactinomas (13.5 ± 1.2 g/dl vs. 15.1 ± 0.9 g/dl, $p < 0.05$). Anemia (hemoglobin < 13 g/dl) was present in 9 (34.6%) patients, all of them with macroprolactinomas. The degree of anemia was mild (hemoglobin > 11 g/dl) in all patients. No correlation between hemoglobin and serum prolactin was found. Hemoglobin concentration was significantly lower in men with hypogonadism ($n=14$) than in eugonadal men. Hemoglobin value was also significantly lower in patients with total hypopituitarism in comparison with subjects with partial hypopituitarism (12.4 ± 1.0 g/dl, $n=7$ vs. 14.0 ± 1.2 g/dl, $n=13$ $p=0.007$). The number of affected pituitary axes was found to be related with the presence of anemia. Logistic regression analysis showed that anemia was related with FT4 (OR 0.23; 95% C.I. 0.06-0.81, $p=0.02$), cortisol (OR 0.81; 95% C.I. 0.68-0.96, $p=0.02$) and the presence of hypopituitarism (OR 20.0; 95% C.I. 1.68-238.63, $p=0.02$). In conclusion, anemia was found in about a third of men with prolactinomas. Our results also suggest that the presence of anemia in these patients seems to be associated with panhypopituitarism.

Nothing to Disclose: PI, JCC, JJD

Pub # P3-307

Session Information POSTER SESSION: CLINICAL - Prolactinomas, Thyrotropinomas (1:30 PM-3:30 PM)

Title Empty Sella with Hyperprolactinemia in a Patient with Sickle Cell Anemia with Multiple Transfusions

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Body Objective: To present a case of endocrine hyperfunction in a patient with Sickle Cell Disease (SCD) with multiple transfusions.
Methods: We present the clinical course, laboratory methods, results, and findings in our patient with SCD relating to her endocrine dysfunction. The related literature is also reviewed.
Results: A 41 y/o woman with PMH of SCD requiring multiple transfusions, cholelithiasis, amenorrhea and galactorrhea for 2 yrs, presented with acute chest syndrome.
Labs done by chemiluminescence were significant for serum ferritin 708.9 ng/ml (10-232), serum prolactin 102 ng/dl (3.0-30.0), IGF-1 59 ng/ml ((90-360), LH 1.48 IU/L (0.61-16.30), TSH 0.998 mIU/L, ACTH, FSH and serum cortisol were within normal limits while estradiol by extraction immunoassay was 21.46 pg/ml (20-150).
MRI of the sella showed partial empty sella with no evidence of pituitary tumor but with a 1.8 cm cystic lesion near the clivus. Literature review reveals that chronic transfusion therapy leads to whole body iron overload subsequently causing organ injury via iron mediated cellular oxidative damage and that endocrine dysfunction is the earliest toxic effect seen in iron overload patients. In our patient, hyperprolactinemia could be due to a stalk effect of the cystic lesion near the clivus. Acutely, chest wall pain may increase serum PRL, however, this patient had amenorrhea/galactorrhea for at least 2 years. Multiple vaso-occlusive episodes due to repeated sickling could lead to pituitary infarction and empty sella, as suggested by Soliman et al., who also reported frequent low IGF-1 levels in SCD patients with empty sella. If the hyperprolactinemia in our patient were due to the periclavial cystic lesion, the IGF-1 level would be less likely to be affected. Also loss of function in the hypothalamic-hypophyseal tract due to iron deposition or infarction could result in loss of dopamine flow to the pituitary allowing a passive increase in prolactin secretion.
Conclusions: Patients with SCD with multiple transfusions are at high risk of endocrine organ failure due both to iron-mediated cellular oxidative damage and infarction due to vaso-occlusive episodes associated with intravascular sickling. They should be monitored lifelong for these complications of SCD and its treatment.

Nothing to Disclose: SC, SN, AS, GB

Pub # P3-308

Session Information POSTER SESSION: CLINICAL - Prolactinomas, Thyrotropinomas (1:30 PM-3:30 PM)

Title Risk of Hypercoagulability in Patients with Prolactinoma Compared to Non-Prolactinoma Pituitary Adenoma

Author String S Yu Mon, A Alkabbani, A Hamrahian, L Kennedy, R Weil, V Makin, D Krupa, J Thornton, D Brennan, BA Hatipoglu
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Body **Objective:** An association between high prolactin levels and increased risk for deep vein thrombosis (DVT), ischemic stroke (CVA) and pulmonary embolism (PE) during pregnancy and in patients on antipsychotic drugs has been suggested in retrospective studies. Hyperprolactinemia increases platelet aggregation via ADP stimulation *in vitro* and *in vivo*. In this study, we compared the incidence of DVT, PE, and ischemic stroke in prolactinoma patients vs. non prolactinoma pituitary adenoma.

Methods: The data were extracted from a prospectively maintained pituitary database with 204 prolactinoma patients (G1) and 81 patients with other pituitary tumors (G2). In G1, 54 patients underwent surgery (all transphenoidal). All the patients in G2 were non-functional pituitary adenoma and 50 of these 81 patients underwent transphenoidal surgery.

Results: The highest prolactin levels (interquartile range) in G1 and G2 were 125 (61.8, 468.5) and 14 (6.8, 26), $P < 0.001$ respectively. Within the prolactinoma patients, those who underwent surgery for different indications had significantly higher initial prolactin levels [206 (96.4, 613) vs. 109.6 (56.5, 388), $P = 0.035$]. The total number of DVT/PE/CVA for the prolactinoma group versus the control group was not statistically significant [8 (3.9%) vs. 2 (2.5%), $P = 0.73$]. Among 8 patients in G1 with an event 4 (2.7%) were in the non surgical group and 4 (7.4 %) in the surgical group ($P = 0.21$). There were 3 DVT's and one CVA in medical group, one DVT one PE and 2 CVA's in surgical group. The 2 events (2.5%) seen in control group were one PE and one DVT , both in the surgical treatment arm.

Discussion: Hyperprolactinemia has been linked to a possible increased risk of events associated with hypercoagulability. We found no significant difference in the frequency of such events in patients with prolactinoma compared to the control group.

Conclusions: Our data do not support the view that hyperprolactinemia *per se* predisposes to hypercoagulability-associated events. Sample sizes may have been too small to detect potential differences. Future studies are warranted.

Nothing to Disclose: SYM, AA, AH, LK, RW, VM, DK, JT, DB, BAH

Pub # P3-309

Session Information POSTER SESSION: CLINICAL - Prolactinomas, Thyrotropinomas (1:30 PM-3:30 PM)

Title High Prevalence of Radiological Vertebral Fractures in Patients with Prolactin-Secreting Pituitary Adenomas

Author String G Mazziotti, T Mancini, M Mormando, E De Menis, A Bianchi, T Porcelli, M Doga, PP Vescovi, L De Marinis, A Giustina
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Body Hyperprolactinemia may cause bone loss but data on fractures are scanty. The aim of this study was to evaluate the prevalence of vertebral fractures in men and women with prolactin(PRL)-secreting adenoma. In this cross-sectional study, 110 patients (78 F, 32 M, median age 46 years, range: 20-81) with PRL-secreting pituitary adenoma (76 with microadenoma and 34 with macroadenoma) and 220 control subjects, with normal PRL values and with comparable age and sex to patients with hyperprolactinemia, were evaluated for vertebral fractures by a morphometric approach and for bone mineral density (BMD) by a dual-energy X-ray absorptiometry at lumbar spine. Vertebral fractures were shown in 37 patients with PRL-secreting adenoma (33.6%) and in 25 controls (11.4%, $p<0.001$). Fractured patients were significantly older ($p<0.001$) and had lower BMD T-score ($p<0.001$), longer duration of disease ($p<0.001$), higher serum PRL ($p<0.001$) and lower serum IGF-I ($p=0.001$) values as compared to patients who did not fracture. Fractures occurred more frequently ($p=0.002$) in patients with untreated hyperprolactinemia vs. patients treated with cabergoline whose frequency of vertebral fractures was comparable to that observed in control subjects. Males and females with prolactinomas showed no significant difference in BMD ($p=0.4$) and prevalence of vertebral fractures ($p=0.6$). Logistic regression analysis demonstrated that duration of disease maintained a significant correlation with vertebral fractures even after correction for age, treatment with cabergoline, BMD, serum IGF-I and serum PRL values. In conclusion, hyperprolactinemia is associated with high prevalence of radiological vertebral fractures in men and women with PRL-secreting adenoma.

Nothing to Disclose: GM, TM, MM, EDM, AB, TP, MD, PPV, LDM, AG

Pub # P3-310

Session Information POSTER SESSION: CLINICAL - Prolactinomas, Thyrotropinomas (1:30 PM-3:30 PM)

Title Glucose Metabolism, Insulin Sensitivity, Markers of Inflammation and Adiponectin Levels in Hyperprolactinemic Patients Who Have Long-Term Normal Prolactin Levels by Treatment with Dopamine Agonists

Author String M Medic-Stojanoska, N Curic, S Pekic, B Kovacev-Zavistic, S Nikolic, B Vukovic, M Donkic, J Novakovic-Paro, V Popovic-Brkic
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Body Data obtained in human and experimental studies suggest that hyperprolactinemia may cause disturbances in glucose metabolism by stimulating insulin release, enhancing peripheral insulin resistance, increasing the level of inflammation and by changing adipokine release. Recent studies show beneficial effect of bromocriptine on glucose homeostasis in obese type 2 diabetic patients (1).
The aim of this study is to evaluate glucose metabolism, insulin sensitivity, markers of inflammation and adiponectin levels in hyperprolactinemic patients who have normalized prolactin levels for a long period by dopamine agonist treatment.
Methods: Twenty five hyperprolactinemic non-obese premenopausal women with prolactinoma were recruited and 16 healthy women were included as controls. Patients were treated with dopamine agonists (Bromocriptine or Cabergoline) for 50,7 +/- 58,0 (from 20 to 228) months and long-term normoprolactinemia was achieved. Glucose metabolism was evaluated by oral glucose tolerance test (OGTT). Insulin and C-peptide were measured by Bayer Advia Centaur XP, CLIA. Insulin sensitivity and β -cells secretion were calculated by using specific equations. For the insulin sensitivity index (ISI) (2) and β -cells secretion (2). Prolactin (PRL) mean value of two measurements (Bayer Advia Centaur XP, CLIA), C-reactive protein (CRP) (Immuno-turbidimetric test, Olympus), interleukin (IL)-6 (Roshe, ECLIA) and adiponectin levels (Human Adiponectin/Acrp30 Immunoassay-enzyme) were measured.
Results: Serum levels of PRL were normalized (11,6 +/- 7,76 vs 8,58 +/- 2,05 ng/ml, P=0,15). Glucose (P=0,84, P=0,13), insulin (P=0,07, P=0,24) and C-peptide levels (P=0,06, P=0,27) during OGTT were similar in patients and controls. Calculated ISI and calculated index of β -cells secretion did not differ significantly between patients and controls. Markers of inflammation (CRP and IL-6) were in the normal range and similar in both groups (P=0,16; P=0,99). Adiponectin was in the reference range and similar to controls (P=0,47).
Conclusion: Long-term normoprolactinemia in previously hyperprolactinemic patients achieved by chronic dopamine agonist treatment was associated with normal glucose metabolism, insulin sensitivity, markers of inflammation and adiponectin levels. It remains to be elucidated whether this is due to normalized prolactin levels or the action of dopamine agonists per se!

(1) Pijl H et al., Diabetes Care 2000; 23:1154-1161

(2) Nathan DM et al., Diabetes Care 2001; 24(4):796-797

Nothing to Disclose: MM-S, NC, SP, BK-Z, SN, BV, MD, JN-P, VP-B

Pub # P3-311

Session Information POSTER SESSION: CLINICAL - Prolactinomas, Thyrotropinomas (1:30 PM-3:30 PM)

Title Development of Impulse Control Disorders in Patients with Dopamine Agonist-Treated Prolactinomas: A Case Series

Author String MR Nannenga, PJ Tebben, TB Nippoldt
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Body

Background
Impulse control disorders (ICDs) comprise a broad range of entities such as hypersexuality, pathologic gambling, and compulsive spending that share a common venality--the inability to resist urges, frequently subjecting the sufferer to a detrimental life-altering course. An increased prevalence of ICDs has been reported in various disease entities treated with dopamine agonists (DAs). To date, there are 2 case reports describing the development of ICDs in patients with DA-treated prolactinomas. We report 3 patients with prolactinomas who developed ICDs on DA therapy.

Case 1
A 51 yr old male was diagnosed with a 2.7-cm macroprolactinoma (Prolactin (P) 1284 ng/mL) and placed on bromocriptine (B) 25 mg/d. Within 5 months, he developed new hypersexuality and began gambling compulsively. During 8 years of DA use, he accumulated \$150,000 in ICD-related debt. At age 59, his DA was discontinued, with prompt resolution of all ICDs.

Case 2
A 14 yr old male with headaches was diagnosed with a 2.8-cm macroprolactinoma (P 5168 ng/mL). He took B 22.5 mg/d for 3 yrs before switching to cabergoline 2.25 mg/wk for 4 yrs. Within two yrs of B initiation, he developed hypersexual and promiscuous behaviors that contributed to a volatile relationship with his long-term partner, culminating in 2 failed suicide attempts. His ICD resolved within months of DA discontinuation at age 21.

Case 3
A 36 yr old male with bitemporal hemianopsia was diagnosed with an invasive 2-cm macroprolactinoma (P 6400 ng/mL). After surgical debulking, he took B 20 mg/d for 10 yrs, later switching to pergolide 0.15 mg/d for insurance purposes. After 20 yrs of DA therapy, he was queried regarding any history of ICDs. He revealed that a casual gambling habit had transformed into a marriage-threatening addiction several yrs prior while he was taking B 20 mg/d, and spontaneously abrogated when he switched to pergolide.

Discussion
The apparent precipitation of ICDs by DAs in patients with prolactinomas supports the notion that dopaminergic agents predispose to ICDs in a disease-independent manner. Preliminary investigation in patients with DA-related ICDs utilizing functional PET scanning suggests a paradoxical decrease in cerebral reward/punishment center activity.

Conclusion
Screening for ICDs should be undertaken in all patients with DA-treated prolactinomas. Prospective investigation to characterize the incidence and potential predisposing factors is warranted.

Nothing to Disclose: MRN, PJT, TBN

Pub # P3-312

Session Information POSTER SESSION: CLINICAL - Prolactinomas, Thyrotropinomas (1:30 PM-3:30 PM)

Title Hypersexuality in Cabergoline-Treated Prolactinoma

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Body Background: Prolactinomas are the most common type of secretory pituitary tumors and are medically treated with Dopamine agonists that decrease prolactin, shrink tumor size, reverse gynecomastia and improve sexual dysfunction. Headache, dizziness and gastric upset are the most common side effects of cabergoline treatment Behavioral disorders- pathological gambling, compulsive shopping, obsessive behavior and hypersexuality resulting from cabergoline therapy have been rarely described in the literature. We report a case of a 61 year old male who presented with symptoms of hypersexuality with long term and high dose cabergoline use. Clinical Case: A 61 year old male presented to the endocrine clinic with behavioral changes while on cabergoline. Eighteen years ago, the patient was diagnosed to have a 13x11x15mm prolactin secreting pituitary macroadenoma abutting the lateral wall of the left cavernous sinus. He developed visual field changes and was prescribed bromocriptine without benefit. He was then switched to increasing doses of cabergoline and his symptoms improved only at a very high dose of 1gm/day. He began noticing behavioral changes of hypersexuality, extramarital affairs and compulsive sexual behaviors that created tension in his married life and led to the need for marital counseling. These symptoms persisted even on decreasing the dose of cabergoline to 0.5mg/day. Six weeks after stopping cabergoline, MRI showed no change in the size of his tumor when compared with an MRI done 1 year ago, prolactin levels remained low at 1.2ng/ml(2-14ng/ml) with normal visual fields and the patient noticed a dramatic improvement in his behavioral symptoms. Discussion: To our knowledge, only one case of hypersexuality has been reported previously in a patient treated for prolactinoma. Hypersexuality has been witnessed more commonly in patients being treated with high dose of cabergoline for parkinson's disease. It is felt that this side effect is dose related. Our patient was being treated with 1mg/day of cabergoline which is similar to the dosing used in parkinson's disease. These abnormal behaviors are reversible with discontinuation of therapy. It is important that physicians who treat patients with dopamine agonists recognize these pathological symptoms that masquerade as psychiatric illnesses.

Disclosures: RSZ: Speaker, Novo Nordisk; Bristol-Myers Squibb; Astra Zeneca. Nothing to Disclose: AG

Pub # P3-313
Session Information POSTER SESSION: CLINICAL - Prolactinomas, Thyrotropinomas (1:30 PM-3:30 PM)
Title Aripiprazole for Dual Treatment of Psychosis and Prolactinoma: A Case Report
Author String T Moin, W Dunn, R Espinoza, A Van Herle, S Korenman
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Body **Background:** Iatrogenic hyperprolactinemia is a side effect of many antipsychotic medications that block dopamine-type 2 receptors (D2Rs). Rarely, patients with psychosis and mood disorders can present with tumorogenic hyperprolactinemia. Aripiprazole is a new atypical antipsychotic medication that partially blocks D2R and lowers prolactin levels.[i] The exact mechanism remains unclear but receptor occupancy time, potency, and differential signaling through short and long D2Rs may play a role.[ii] Thus, aripiprazole may be ideal for treating psychosis in the setting of tumorogenic or iatrogenic hyperprolactinemia. [iii]
Clinical Case: A 60-year old woman was admitted with chronically worsening psychosis including auditory hallucinations and persecutory delusions. She had no known past medical or psychiatric history and was taking no medications. She complained of headaches but denied visual changes or galactorrhea. Head CT showed a 2.1 x 1.5 cm mass in the sella turcica. Pituitary MRI showed a partially hemorrhagic 1.6 x 1.6 x 1.5 cm pituitary mass, without cavernous sinus invasion or compression of the optic chiasm. Laboratory results revealed an elevated prolactin of 312 ng/mL (normal range 3.0-23.1 ng/mL). LH and FSH were suppressed at 1.0 mIU/mL (post-menopausal range 16-63) and 2.5 mIU/mL (post-menopausal range 21-106) respectively and the remainder of the pituitary evaluation was normal. Aripiprazole was initiated at 2.5mg daily and gradually titrated to 30mg daily with improvement in psychiatric symptoms. On hospital discharge 2 weeks later, prolactin was decreased to 65.0 ng/mL. On outpatient follow-up 1 month later, prolactin was down to 31.1 ng/mL.
Conclusion: Unlike many other antipsychotic medications, aripiprazole is a partial dopamine agonist that lowers prolactin levels. This case is the 4th case report in the literature demonstrating the therapeutic role for aripiprazole in treating patients with psychosis and tumorogenic hyperprolactinemia. Aripiprazole may also be of use in cases of dopamine agonist intolerance or failure but further studies are needed to elucidate safety in patients without a psychiatric illness.

[i] Wood M et al.,Expert Opin. Investig. Drugs 2007; 16(6):771-775

[ii] Hoffer ZS et al.,Psychosomatics 2009; 50(4):317-324

[iii] Keck PE et al.,Expert Opin. Investig. Drugs 2003; 12(4):655-662

Nothing to Disclose: TM, WD, RE, AVH, SK

Pub # P3-314

Session Information POSTER SESSION: CLINICAL - Prolactinomas, Thyrotropinomas (1:30 PM-3:30 PM)

Title Prolactinomas Diagnosed in Menopause

Author String C Ballarino, S Diez, G Pensi, V Schmoll, G Stalldecker
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Body INTRODUCTION: prolactinomas are the most frequent pituitary adenomas in fertile women; this does not appear to be the same in menopausal women, although information on this subject is limited. As microprolactinomas are asymptomatic in this group, it is difficult to establish the true frequency. OBJECTIVE: to determine the occurrence and characteristics of prolactinomas and hyperprolactinemia in our group of menopausal women. MATERIAL AND METHODS: The medical histories of 70 women with diagnosis of prolactinomas during the period 2001-2010 were assessed, 66 diagnosed in premenopause (group 1) and 4 diagnosed in menopause (group 2). On the other hand, we evaluated 100 asymptomatic menopausal women (group 3) to whom prolactin (NV: 5-25 ng/dl) had been requested as a hormonal routine control of menopause. All serum samples were analyzed by direct chemiluminescence ACS 180. RESULTS: In group 1 there were 40 microprolactinomas and 30 macroprolactinomas, and the mean age at diagnosis was 30.8 +/- 10.2 years. In group 2 we found 3 macroprolactinomas (age 60,52 and 60). The median of prolactin was 583.6 ± 307.2 ng/ml. These patients had a history of amenorrhea of 18, 20 and 25 years and the FSH was 67, 14 and 0.6 mUI/ml at diagnosis, respectively. A microprolactinoma was diagnosed in a 50 year old woman who had been hysterectomized 12 years before and FSH was 43 mUI/ml at diagnosis, the basal prolactin level in this patient was 190 ng/dl. All patients of this group presented amenorrhea without climacteric syndrome and had history of fertility before the age of 30. The relationship between prolactinomas diagnosed before menopause and during menopause was 16.5 to 1. Asymptomatic menopausal women (group 3) presented a mean age of 50 +/- 4 years, with mean of 4 +/- 3 years of menopause and a median FSH of 73.3 mUI/ml. Of 100 examined patients, 2 (2%) had hyperprolactinemia: 1 was drug induced and the other was a macroprolactinemia. The remaining 98 patients were normoprolactinemic with a median of 8.4 (IC 95 %: 8 - 9.7 ng/dl). CONCLUSIONS: 1- We have not detected prolactin levels that would suggest the presence of prolactinomas in the group of asymptomatic menopausal women. 2- Prolactinomas appear to be uncommon in menopause, or at least, infrequently diagnosed. 3- The presence of macroprolactinomas in our menopausal women could reflect the natural growth of lately diagnosed tumors.

Nothing to Disclose: CB, SD, GP, VS, GS

Pub # P3-315

Session Information POSTER SESSION: CLINICAL - Prolactinomas, Thyrotropinomas (1:30 PM-3:30 PM)

Title Macroprolactinomas and Pregnancy: Analysis of a 38-Women Cohort Treated by Dopamine Agonists

Author String B Decoudier, G Raverot, D Ancelle, A-C Hecart, F Borson-Chazot, B Delemer
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Body Pregnancy is associated with an increased risk of tumor growth in macroprolactinoma leading to discuss surgery in such cases.
Objectives: impact of pregnancy on prolactin secretion and tumor growth in a cohort of women with macroprolactinoma, including large tumors > 20 mm, and medically treated.
Methods: retrospective study of all the women with macroprolactinomas diagnosed in two university hospitals, treated by dopamine agonists, with at least one pregnancy after diagnosis.
Results: 38 women, 82 spontaneous pregnancies, 64 children for 35 women.
On diagnosis: mean age 23 years, median prolactin level 305 [mu]g/l (90-7804), prolactin level [ge] 500 [mu]g/l in 15 women, median tumor size 15 mm (size [ge] 20 mm in 12 cases), abnormal visual field in 7 cases. Before pregnancy, 16 patients received cabergoline, 20 bromocriptine, 2 quinagolide. Prolactin level was controlled in 37 women. Visual troubles disappeared totally in 6 patients, partially in one.
Residual tumor was not visible on RMI in 10 patients, [ge] 10 mm in 11 (maximum 23 mm). During pregnancy, medical treatment was stopped in 20 patients (5 adenomas [ge] 20 mm). 8 patients had symptomatic (5 headache, 3 visual troubles) tumor evolution which was controlled by dopamine agonists either reintroduction or dose increase in 7 patients. Surgery was mandatory in one case of apoplexy during pregnancy (second trimester) and one immediately post-partum.
Prolactinoma size at diagnosis, residual size after medical treatment and treatment stop during pregnancy were not statistically significant risk factors of tumor enlargement.
Conclusion: This study, performed in a large cohort, confirms that pregnancy is a risk period of symptomatic macroprolactinoma enlargement (21%) and apoplexy but no specific risk factor for adenoma growth was found. Medical treatment is able to control tumor enlargement in almost all the cases except apoplexy which requires surgery. Macroprolactinomas, even large ones, do not seem to require debulking before pregnancy.

Nothing to Disclose: BD, GR, DA, A-CH, FB-C, BD

Pub # P3-316
Session Information POSTER SESSION: CLINICAL - Prolactinomas, Thyrotropinomas (1:30 PM-3:30 PM)
Title A Rare Occurrence of Extensive Spherical Amyloid Deposits in a Prolactin-Secreting Pituitary Macroadenoma
Author String S Ishaq, JD Wilson, A Nanda, SN Levine
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Body

Introduction

Pituitary adenomas are the most common tumors of the sellar region, but the occurrence of extensive spherical amyloid deposits in a pituitary adenoma is rare. Less than 20 cases have been reported, almost exclusively in prolactinomas.

Case Report

A 45-year old man presented with a history of a prolactin-secreting pituitary macroadenoma diagnosed 18 months earlier when he developed complaints of galactorrhea, erectile dysfunction and fatigue. His prolactin level was 2,470 ng/ml. Total and free testosterone, FSH, LH and IGF-1 levels were low, while TSH and free T4 were normal and the hypothalamic-pituitary-adrenal axis was intact. A pituitary MRI showed a 3 x 3 x 3 cm lobulated, irregular pituitary mass, invading the sphenoid and cavernous sinuses, abutting the optic chiasm superiorly. It had low intensity on T2-weighted images, heterogeneous intensity on T1-weighted images, with slight contrast enhancement of the margins in a ring-like fashion. His visual field examination showed a right superior homonymous hemianopsia. After 18 months of treatment with cabergoline up to 1 mg twice weekly, the prolactin level decreased to 33 ng/ml but the pituitary macroadenoma decreased only minimally in size to 2.8 x 2.7 x 2.5 cm. The patient underwent trans-sphenoidal resection of the pituitary macroadenoma. Calcification of the tumor was noted intra-operatively. The pathology of the sellar mass on H&E stained sections demonstrated the presence of very sparse small nests of pituitary adenoma interspersed within abundant pale eosinophilic material arranged in round, spherical concentric lamellated structures of variable size. In many areas, the eosinophilic material was confluent and partially calcified with a "salmon orange" color on light microscopy and "apple-green" birefringence on polarized light. The sparse neoplastic adenohypophyseal cells showed positive immunohistochemical staining for chromogranin, synaptophysin and prolactin.

Conclusion

The MR imaging characteristics of spherical amyloid deposits in a pituitary adenoma have been described only twice. Low intensity on T2 weighted images and minimal contrast enhancement are features of amyloid deposits in pituitary tumors. These findings should alert endocrinologists that these prolactinomas may not respond to dopaminergic agonists with a significant decrease in tumor size and neurosurgeons that the tumor may be atypical indicating the presence of amyloid and calcifications.

Nothing to Disclose: SI, JDW, AN, SNL

Pub # P3-317

Session Information POSTER SESSION: CLINICAL - Prolactinomas, Thyrotropinomas (1:30 PM-3:30 PM)

Title Voxel-Based Volumetric Assessment in Long-Term Treated Giant Prolactinomas

Author String AC Borges-Leal, AC Santos, M de Castro, AC Moreira, PCL Elias
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Body Retrospective study to evaluate voxel based volumetric assessment in long-term treated giant prolactinomas (>4,0cm and PRL >2,000ng/ml). From 1996-2010, among 95 micro and macroprolactinomas, 11 patients with giant prolactinoma were identified (8 men, 3 woman aged 16-57 years, median 44yrs). Direct 3D tumor volume from MRI studies was assessed before dopamine agonist (DA) treatment in seven patients, and in all patients during follow-up. All but one patient started bromocriptine (BCP), with increasing dose as necessary to up to 15mg/day. Cabergoline (CBG) was used as first line treatment in one patient and as second line treatment in five patients (starting dose of 1.5mg/week increased up to 5mg/week). Patients were followed by hormone measurements, sellar MRI, and visual examinations. Median follow-up duration was 54 months (20-164 months). Mean serum PRL before treatment was 10461±9087ng/ml (range 3079-31200; normal 5-25ng/ml) and significantly decrease to 143±225ng/ml (range 1.8-754) (P<0.05) after treatment. Levels normalized in five patients within 1.5-64 months and decreased 2-3 times of normal in three. The other three showed significant decreased between 88 and 97% in PRL levels. Pre-treatment tumor volume was 47.7±17cc using ellipsoid volume formula ($VolElip=height*length*width*[pi]/6$) and 31±16cc assessed by 3DVol. Mean maximal volume reduction after treatment was 70±27% (range 13-99%) and 73±16%(range 52-96%) considering VolElip and 3DVol, respectively. All patients reduced tumor volume more than 50% by 3DVol while two patients did not achieve 50% volume reduction using VolElip. Mean 3DVol reduction in the first year was 62%. Ten patients had visual field defects at diagnosis, and improved in seven. Giant prolactinoma appears to be a rare entity more prevalent in men. Hypogonadism symptoms neglected may account for its higher prevalence in men. DA represents the first-line therapy effective in reducing PRL and determining tumor shrinkage. Although CBG is the first drug used in macroprolactinomas, one must point out the higher and longer doses needed and its potential involvement in cardiac valve dysfunction. BCP remains effective in a significant number of patients with giant prolactinomas Assessing tumor volume using VolELip could misclassified some patients as DA resistant. 3DVol represents a useful and more accurate tool in monitoring prolactinoma treatment response to DA.

Sources of Research Support: FAPESP Grant 07-58365-3.

Nothing to Disclose: ACB-L, ACS, MdC, ACM, PCLE

Pub # P3-318

Session Information POSTER SESSION: CLINICAL - Prolactinomas, Thyrotropinomas (1:30 PM-3:30 PM)

Title Determining Efficacy of Dopamine Agonist Treatment for Prolactinomas Utilizing Novel MRI Techniques

Author String AE Reiher, TA Gallagher, PA Turski, JS Kuo
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Body

Introduction:
First-line treatment for prolactinomas is dopamine agonists, but there is little evidence in the literature regarding expected changes seen on MRI that correlate with serum prolactin levels. This study will define these MRI changes utilizing novel MRI techniques such as PROPELLER and correlate them with serum prolactin levels over the course of treatment.

Methods:
IRB approval for a retrospective review was obtained. Inclusion criteria: macroprolactinoma, no other hormonal pituitary hypersecretion, MRI available for viewing in our PACS system. Serum prolactin levels were correlated with changes seen on pituitary protocol MRI over time. As skull base artifact often limits conventional MRI, PROPELLER T2 and diffusion weighted imaging were employed to optimize the difficult to-image sellar region. Detailed MR evaluation included: change in tumor volume, development of hemorrhagic or cystic components, cavernous sinus invasion, diffusion and apparent diffusion coefficient (ADC) characteristics, and T1- and T2-weighted signal changes. Symptoms, visual field deficits, and any associated hypopituitarism were included. Surgically treated patients were followed as a control group.

Results:
Medically treated patients had variable response to dopamine agonist therapy; however, preliminary data suggest a positive response to treatment is associated with increased ADC and T2 signal intensity. Patients subsequently treated with surgical resection of prolactinomas were found to have no recurrence after an average of 17 months follow-up.

Conclusions:
Using novel MR techniques in adjunct to serum prolactin levels, we can determine the expected evolution of a treated prolactinoma, prognostic imaging findings, as well as an optimal imaging follow-up regimen. We may also be able to predict which patients will fail medical therapy based on the initial MR appearance of the tumor. This will be both cost-effective by reducing unnecessary additional MRIs, and provide a new way to monitor the course of a prolactinoma over time.

Nothing to Disclose: AER, TAG, PAT, JSK

Pub # P3-319

Session Information POSTER SESSION: CLINICAL - Prolactinomas, Thyrotropinomas (1:30 PM-3:30 PM)

Title Tumor Disappearance Following Individualized High-Dose Cabergoline Therapy for Prolactinomas: Association with Posttreatment Prolactin Values

Author String M Ono, N Miki, R Makino, T Seki
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Body
Context: Cabergoline normalizes hyperprolactinemia and reduces tumor size in a majority of patients with prolactinomas. Tumor shrinkage usually follows serum prolactin (PRL) suppression, but tumor disappearance occurs only in a minority (15-26%) of patients.
Objective: We conducted individualized high-intensity cabergoline therapy in 150 patients with prolactinomas and prospectively evaluated tumor regression on magnetic resonance imaging and assessed parameters associated with tumor disappearance 1 and 2 years after treatment.
Methods: Patients consisted of 114 women and 36 men (60 macro- and 90 microadenomas). Seventy-one and 22 patients had received prior bromocriptine or surgical therapy, respectively. Adenomas that had unclear margins were not included in this study. Cabergoline was started at a standard dosage and schedule and the highest daily dose was set at 3 mg/day. The dose was increased step-wisely on the basis of patients' PRL responsiveness and was fixed at the time of PRL normalization as described (JCEM 93: 4721, 2008). Twenty-three patients, who became pregnant after 1 year, were not included in an analysis at 2 years.
Results: Cabergoline normalized hyperprolactinemia in all 150 patients after 1 year, and post-treatment PRL levels remained normal in all 127 patients treated for up to 2 years. Mean cabergoline dose was 2.2 ± 0.2 (range, 0.5-12) mg/week. AIC analysis revealed a close relationship of tumor disappearance with post-treatment absolute PRL values, measured at 6 to 12 months, but not with other parameters including prior medical or surgical therapy, cabergoline dose, tumor size, pretreatment PRL, gender, age at diagnosis, and length of time to PRL normalization. There was a strong negative correlation between the posttreatment PRL value and the degree of tumor shrinkage ($r = -0.850$, $P < 0.0001$). Stratified analysis of the post-treatment PRL showed that in patients with low-normal PRL of <2.76 [mu]g/L, tumor disappeared in 54.3% of 81 patients by 1 year and in 97.4% of 76 patients by 2 years, whereas in patients with high-normal PRL of 9.66-15.0 [mu]g/L, tumor did not disappear in any of 43 patients by 1 year or in any of 30 patients by 2 years.
Conclusion: To achieve high prolactinoma disappearance rate with cabergoline, an aggressive therapy to suppress hyperprolactinemia to low-normal levels appears prerequisite instead of merely normalizing tumoral hyperprolactinemia.

Nothing to Disclose: MO, NM, RM, TS

Pub # P3-320

Session Information POSTER SESSION: CLINICAL - Prolactinomas, Thyrotropinomas (1:30 PM-3:30 PM)

Title Are Stable or Decreasing Prolactin Levels in Patients with Prolactinoma a Surrogate Marker for Lack of Tumor Growth?

Author String AG Alkabbani, SY Mon, B Hatipoglu, L Kennedy, C Faiman, R Weil, A Hamrahian
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Body

Introduction: Optimal follow up imaging interval for patients with prolactinomas treated with dopamine agonists (DA) or monitored without therapy is unknown, with significant variability in clinical practice. We hypothesize that there is no increase in tumor size in patients with prolactinomas who have a stable or reduced prolactin level.

Methods: We identified 85 patients with prolactinoma since 2001 from a prospectively maintained pituitary database, who had at least two sets of prolactin levels and pituitary MRIs, and had their prolactin levels and MRIs within 3 months interval. Patients with prolactin levels <200 ng/mL on drugs known to cause hyperprolactinemia were excluded.

Results: The median (25th - 75th) age and sex ratio (F/M) were 46 (36 - 56) years and (57.7%/42.3%), respectively. Among 74 patients treated with DA, 63 (85.1%) were receiving cabergoline, mean weekly dose 1.13 mg; 10 (13.5%) bromocriptine, mean daily dose 8 mg; 1 (1.3%) pergolide, mean daily dose 0.1 mg. The rest (n= 11, 12.9%) were observed off DA.

The prolactin level (ng/mL) and tumor size (mm) at the time of initial and latest MRI study were 136 (61.7 - 649.3), 13 (7.0 - 22.6) and 13.4 (4.8 - 42), 11 (5 - 18.3), respectively, with a time interval of 31.2 (19.2 - 55.2) months. The tumor size at the time of initial MRI was micro- in 35.3%, macro- in 58.8% and the rest (5.9%) had no visible tumor. Forty-seven (55%) patients had a decrease in tumor size and the rest except one with evidence of pituitary hemorrhage, had stable tumor size. Twenty-one patients underwent pituitary surgery due to lack of response or intolerance of DA.

Discussion: Excluding patients with pituitary hemorrhage, we could not identify any patient in our study who had an increase in tumor size during follow up when their prolactin levels were reduced or stable regardless of their treatment with DA. Imaging studies in such patients are mainly warranted to assess tumor shrinkage in response to DA or when a pituitary apoplexy is suspected.

Conclusion: Tumor growth in patients with prolactinoma with a stable or decreasing prolactin level should be a very rare event and does not necessitate routine frequent pituitary imaging.

Nothing to Disclose: AGA, SYM, BH, LK, CF, RW, AH

Pub # P3-321

Session Information POSTER SESSION: CLINICAL - Prolactinomas, Thyrotropinomas (1:30 PM-3:30 PM)

Title Management of a Woman with Giant Invasive Prolactinoma and Pituitary Apoplexy -- A Multidisciplinary Team Effort

Author String N Stojanovic, K Gunganah, M Vidyarti, S Chawda, Z Khatami, J Pollock
Queen's Hospital, Romford, London, UK; Queen's Hospital Romford, London, UK; Queen's Hospital, Romford, London, UK; Queen's Hospital, Romford, London, UK

Body **Background:** Giant invasive prolactinomas in women are rare and their natural history is not clear. Treatment of these tumors requires a multidisciplinary team.
Case: 52 year old woman was referred to the ophthalmology department with suspected thyroid eye disease. On examination the patient had a frozen left eye, and the visual acuity was 1/60. Thyroid function tests were TSH 4.2mU/l (normal) and free thyroxine 6.7pmol/l (range 10-19.8).
Patient's last menstrual period was 10 years prior to the presentation. Subsequent pituitary tests function ordered by a biochemistry consultant were in keeping with hypopituitarism and prolactin was elevated at >500,000U/l (normal < 460U/l). Thus, an urgent endocrine opinion was sought.
Pituitary imaging revealed a giant pituitary tumor eroding the skull base, invading the left orbit and compressing the brainstem. Treatment options were discussed with the patient. She rejected surgery and was started on a low dose of cabergoline. Her consciousness deteriorated due to a presumed minor tumor haemorrhage a week after starting the treatment and she had emergency tumor debulking. The patient had a prolonged postoperative recovery and was discharged several weeks after the operation. Despite the treatment with cabergoline, three months later she required further tumor debulking after an episode of pituitary apoplexy. The second operation was done through the occipital craniotomy approach.
After the second operation the patient's general condition has significantly improved. Vision in the left eye and eye movements have also improved. She remains well on cabergoline, steroid and thyroxine replacement.
Conclusion: This patient's case emphasizes the need for close multidisciplinary team cooperation in looking after patients with giant invasive prolactinomas. The diagnosis in our patient was made by a biochemist and a close liaison between a neurosurgeon, endocrinologist and ophthalmologist has facilitated the optimal management.
Of note, patient's menstrual history implies that these tumors take some 10 years to develop.

Nothing to Disclose: NS, KG, MV, SC, ZK, JP

Pub # P3-322

Session Information POSTER SESSION: CLINICAL - Prolactinomas, Thyrotropinomas (1:30 PM-3:30 PM)

Title Cabergoline-Resistant Prolactinoma: Failure to Achieve Euprolactinemia and Normal Ovulatory Function Despite High-Dose Cabergoline Therapy

Author String Ck Mamillapalli, E Rico, S Nicoleta, C Fratianni, K Romesh, G Smita
Southern Illinois University School of Medicine, Springfield, IL

Body Ono et al have recently reported normalization of hyperprolactinemia with restoration of ovulatory cycles in all (n=85) patients treated with high dose cabergoline. Eighty patients conceived a total of 95 reported pregnancies. We report a case of cabergoline resistance in a patient who failed to normalize prolactin and remained anovulatory despite cabergoline therapy at a dose of 10 mg/week.
Case: Patient was diagnosed to have secondary amenorrhea at age 18. Two years later her serum prolactin (PRL) was reported elevated at 2000 ng/ml (normal 3-24), and brain MRI showed a pituitary macroadenoma (11 mm x 10 mm). She was treated with bromocriptine and soon switched to cabergoline at 2mg/week due to intolerance. Over the ensuing 17 months her pituitary tumor shrank to 4 x 7 mm, and PRL level fell to 120 ng/ml. She had several interruptions of medical therapy largely related to intermittent nonadherence. At age 26 she was on cabergoline 10 mgs/week for 16 months, and she remained hyperprolactinemic (range: 200 -230 ng/ml) with sporadic menses. Patient was unable to conceive despite trying actively for 2 years at which point gonadotrophin induction was contemplated. At age 28years, she conceived (twin pregnancy) with HCG/Follistim therapy. At conception her PRL was 256 ng/ml with only a small microadenoma on the MRI scan. At 7 weeks gestation cabergoline was discontinued; PRL rose to 414 ng/ml at 11 weeks of gestation. She remained well during pregnancy until 25 weeks gestation when emergency C-section was required for placental abruption. Following delivery only one infant survived. She breastfed this infant for 16 months and he remains well. Repeat pituitary MRI done 2 year postpartum showed small pituitary adenoma. She presently continues to be hyperprolactinemic (495 ng/ml) with persistent galactorrhea/amenorrhea despite tumor shrinkage.
Discussion: This case is one of the very few reported cases where cabergoline dose of > 9 mg/week failed to resolve hyperprolactinemia and anovulation. Furthermore, discordance persisted between the tumor size shrinkage and degree of hyperprolactinemia. Additionally, following initial robust decline in PRL level in response to cabergoline therapy, PRL levels did not rise to pre cabergoline range despite its discontinuation - possibly reflecting loss of tumor volume. Our case also highlights the efficacy of ovulation induction when dopamine agonist therapy has not been successful in cases with persistent hyperprolactinemia.

Ono M et al. J Clin Endocrinol Metab 2010; 95(6):2672-2679.

Nothing to Disclose: CKM, ER, SN, CF, KR, GS

Pub # P3-323

Session Information POSTER SESSION: CLINICAL - Prolactinomas, Thyrotropinomas (1:30 PM-3:30 PM)

Title Dopamine Agonist-Resistant Pituitary Adenoma in a Patient on Long-Term Treatment with Neuroleptics

Author String LK Dzeranova, LY Rozinskaya, EA Pigarova, NS Bykanova, DS Mikhaylova
Endocrinology Research Centre, Moscow, Russian Federation

Body A differential diagnosis of prolactin (PRL)-secreting pituitary adenoma and neuroleptic induced hyperprolactinemia may possess some difficulties.

A 19-year old man was referred to tertiary care center with hyperprolactinemia (PRL - 6936 ME/l) and endosuprasellar pituitary adenoma 13*18*14 mm for further evaluation. He was taking neuroleptic drugs (risperidon and clozapine) for several past years to treat an organic psychiatric disorder (schizophrenia). His main complains were excessive body mass, headaches, loss of libido and gynaecomastia. His testosterone level appeared to be low - 1,7 nmol/l, there was no abnormalities in visual fields.

His therapeutic interventions included a substitution his neuroleptic drugs with the one with low dopamine blocking ability (aripiprazole) and initiation of cabergoline treatment with gradual increase to 3 mg/week. No positive effect was observed through 6 months of treatment (increase in size of pituitary adenoma 16*21*14, persistence of hyperprolactinemia and hypogonadism), which was consistent with dopamine resistant prolactinoma.

The patient underwent transnasal adenomectomy. The histological examination and immunohistochemistry of surgical sample confirmed PRL-secreting pituitary adenoma. His PRL postoperatively fell to 30 ME/l. The patient developed diabetes insipidus that was compensated on 0,6 mg desmopressin per os.

The case of our patient stresses the importance of larger endocrinology workup in patients receiving psychotropic medications that influence dopamine pathways.

Nothing to Disclose: LKD, LYR, EAP, NSB, DSM

Pub # P3-324

Session Information POSTER SESSION: CLINICAL - Prolactinomas, Thyrotropinomas (1:30 PM-3:30 PM)

Title Outcomes after Dopamine Agonist Withdrawal in Macroprolactinomas

Author String JE Sohn, J Menendez, GT Reed, CN Shannon, KO Riley, TB Vaughan
The University of Alabama at Birmingham, Birmingham, AL; The University of Alabama at Birmingham, Birmingham, AL

Body

Background
There is little data regarding outcomes following dopamine agonist withdrawal in macroprolactinomas. A recent meta-analysis found only 9 studies, encompassing a total of 159 subjects, addressing this important clinical issue. In this study, only 16% of subjects with macroprolactinomas achieved persistent normoprolactinemia after withdrawal of dopamine agonists (1). The purpose of this study was to evaluate the rate of remission of hyperprolactinemia after dopamine agonist withdrawal in patients with macroprolactinomas and to assess the predictive value of certain clinical features on outcome.

Methods
We retrospectively reviewed 105 medical records of patients with documented prolactinomas to identify 12 subjects with macroprolactinomas that were withdrawn from dopamine agonists after achieving normoprolactinemia. Patients with a prolactin level above the upper reference range of 29.2 ng/mL were considered to have a recurrence. Time to recurrence was estimated from the time of dopamine agonist withdrawal to the first available elevated prolactin level. As this was a retrospective review, there were no standardized protocols regarding decision to withdraw dopamine agonist therapy, timing of withdrawal, or follow-up after withdrawal. Non-parametric statistics were used to determine the association between several clinical variables and remission status.

Results
Median prolactin level at time of diagnosis was 2600 ng/mL and median tumor maximum diameter was 1.8 cm. The 12 subjects were treated with dopamine agonists for a median of 12 years. Median prolactin nadir after treatment was 5.25 ng/mL. 5/12 (41.7%) of subjects had complete regression of their tumors on imaging 7/12 (58.3%) of subjects remained in clinical remission at a median follow-up time of 30 months. An analysis of potential clinical variables showed a significant association between remission and tumor diameter at the time of withdrawal (P=0.045), but no association with sex, race, dose of dopamine agonist, reason for withdrawal of medication, change in tumor diameter, or initial prolactin level.

Conclusions
Many patients with macroprolactinomas may experience clinical remission after treatment with dopamine agonists. Tumor size at the time of withdrawal may help predict outcomes. In this retrospective study, our findings are limited by data availability and non-standardized practices, but these findings may help guide clinical decision-making and future studies.

(1) Dekkers OM, et al. [ldquo]Recurrence of Hyperprolactinemia after Withdrawal of Dopamine Agonists: Systematic Review and Meta-Analysis.[rdquo] JCEM. 95(1): 43-51, 2010.

Nothing to Disclose: JES, JM, GTR, CNS, KOR, TBV

Pub # P3-325

Session Information POSTER SESSION: CLINICAL - Prolactinomas, Thyrotropinomas (1:30 PM-3:30 PM)

Title Long-Term Remission after Withdrawal of Cabergoline and Bromocriptine Therapy in Patients with Prolactinomas

Author String L Vilar, JL Albuquerque, GR Ibiapina, SP Silva, C Viviane, CO Renata, G Amaro, S Vera
Federal University of Pernambuco, Recife, Brazil

Body

Objective: To assess the proportion of patients with prolactinomas who may persist with normal levels of prolactin (PRL) after interruption of 2 years of treatment with the dopamine agonists cabergoline (CAB) and bromocriptine (BCR) agonists.

Subjects and methods: This is a retrospective study that involved 73 patients with prolactinomas (38 micro- and 35 macroprolactinomas) who were treated with CAB (n=45) or BCR (n= 28), No patients were previously treated by surgery or radiotherapy.

Results: The duration of treatment ranged from 25 to 166 months (mean: 52.2 ± 44.6). The duration of follow-up after DA withdrawal ranged from 8 to 42 months (mean: 21.1 ± 9.2). The rate of patients with persistent normal PRL levels after drug withdrawal was higher with CAB than with BCR both microprolactinomas (39.1 vs. 26.7%; $p = 0.38$) and macroprolactinomas (31.8 vs. 23.1%; $p = 0.40$). However, there was no statistically significant difference between the response to both drugs. Overall, 23 (31.5%) patient persisted with normal prolactin levels after CAB and BCR withdrawal.

Conclusions: Our results confirmed to be worthwhile the dopamine agonists withdrawal in patients with prolactinoma treated for at least 24 months. CAB was more effective than BCR but the difference reached was not statistically significant.

Nothing to Disclose: LV, JLA, GRI, SPS, CV, COR, GA, SV

Pub # P3-326

Session Information POSTER SESSION: CLINICAL - Prolactinomas, Thyrotropinomas (1:30 PM-3:30 PM)

Title TSH-Secreting Pituitary Tumor: A Rare Cause of Thyrotoxicosis

Author String SZ Raheem, V Manuballa, L Ospina
William Beaumont Hospital, Royal Oak, MI

Body

Background: Thyroid-stimulating hormone (TSH) - secreting pituitary tumors account for less than one percent of all pituitary tumors and less than one percent of all causes of thyrotoxicosis. It is often difficult to differentiate thyrotoxicosis due to primary hyperthyroidism from thyrotoxicosis due to a TSH-secreting pituitary tumor. Proper evaluation involves the correlation of history and physical exam findings with laboratory and anatomic study findings. It is essential to differentiate the two clinical entities, as management strategies are entirely different. We present a 29-year-old male who developed thyrotoxicosis secondary to a TSH-secreting pituitary tumor.

Clinical Case: A 29-year-old man presented with palpitations, weight loss despite an increased appetite, anxiety, heat intolerance, and muscle weakness. His TSH level was 5.77, and his free thyroxine and free triiodothyronine were 2.7 and 7.0, respectively. His anti-thyroglobulin antibody and anti-thyroid peroxidase antibody were within normal limits, making it unlikely that the patient had an underlying autoimmune thyroid disease. Radioactive iodine uptake scan showed hypertrapping of both thyroid bilateral lobes. CT scan of the head showed an enlarged pituitary gland (1.2 cm X 1 cm) with mild suprasellar extension, and with no evidence of mass effect. Due to the symptomatic nature of the pituitary tumor, the patient underwent endoscopic transsphenoidal resection. Histological analysis of the resected pituitary mass was positive for TSH and negative for adrenocorticotrophic hormone and prolactin, confirming the diagnosis of TSH-secreting pituitary tumor. Following surgery, the patient's thyroid hormone levels returned to normal and his thyrotoxic symptoms subsided.

Conclusion: Thyrotoxicosis encompasses all forms of thyroid hormone excess. The severity of symptoms is variable between patients and does not always correlate with the level of thyroid hormone elevation. Most patients with TSH-secreting pituitary tumors present with signs and symptoms of thyrotoxicosis and are almost never asymptomatic. Very few patients present with mass effect symptoms from the tumor such as headaches and/or visual field disturbances. First line treatment is removal of the tumor through transsphenoidal resection, leading to restoration of euthyroidism. An increased awareness of this rare entity is important for early diagnosis and management.

Nothing to Disclose: SZR, VM, LO

Pub # P3-327

Session Information POSTER SESSION: CLINICAL - Prolactinomas, Thyrotropinomas (1:30 PM-3:30 PM)

Title Clinical Characteristics and Surgical Outcome in TSH-Secreting Pituitary Adenomas: A Single-Center Experience

Author String N Fukuhara, H Nishioka, H Suzuki, A Takeshita, Y Takeuchi, N Inoshita, T Sano, S Yamada
Toranomon Hospital, Tokyo, Japan; Toranomon Hospital, Tokyo, Japan; Toranomon Hospital, Tokyo, Japan

Body

Introduction
TSH-secreting pituitary tumors (TSHomas) are rare tumors but they are recognized with increasing frequency due to the measurement of TSH level in patients with hyperthyroidism, the ultra sensitive TSH assays and the improvement in pituitary imaging. TSHomas were reported that they were frequently macroadenomas and/or invasive adenomas, and were difficult to treat. The aim of this study is evaluate the clinical features, management, and outcome of a large monocentric series.

MATERIAL AND METHODS:
We retrospectively reviewed records for 54 patients (32 women and 22 men; mean age 46.5 years, range 21-72 years) with TSHomas undergoing surgery at Toranomon Hospital between 2001 and 2010, followed for a period up to 84 months.

Results
Of these, TSH was normal or supranormal ranged from 0.795 to 11.279[mu]IU/ml (mean: 3.315) and hyperthyroidism was found in all but one who had undergone incomplete tumor resection. Mean tumor size was 15.4mm (range 4-43) including 10 microadenoma and 44 macroadenomas. In addition, invasion to the cavernous sinus(Knosp grade 3 or 4) was found in 8 out of 54 patients. Tumor was elastic hard mass in consistency in 40 patients (77%). Among these 54 patients, GH & IGF-1 were elevated associated with acromegaly in nine and PRL was supranormal with hypogonadism in three patients. By immunohistochemistry, only TSH was positive in 13/52 (0.25%), TSH-GH in 20/52 (38%), TSH-PRL in 4/52 (8%), TSH-GH-PRL in 12/52 (23%) whereas TSH was negative in 3 patients. Preoperative medical treatment with somatostatin analogs was performed in 33/52 patients. Thyroid hormone levels were normalized 26/30 patients. A tumoral shrinkage was observed in 16/27 patients. TSS was performed in 54 patients; total tumor removal was achieved in 46 patients (85.2%) and subtotal removals was in six patients. After 1 year, 47 patients (87.0%) met the criteria of surgical favorable outcome. Somatostatin analogs allowed normalization in 4 patients not cured by surgery. A tumor recurrence was found in only one patient over the same period and transient or permanent thyroid hormone replacement was needed in 5 patients.

Conclusion
Ultrasensitive methods for TSH measurement led to an earlier recognition of TSHomas and they are today more frequently found at the stage of microadenomas, and have better surgical results as compared with older series. Additionally, the excellent response to somatostatin analogs has been accompanied by an improvement in the prognosis.

Nothing to Disclose: NF, HN, HS, AT, YT, NI, TS, SY

Pub # P3-328

Session Information POSTER SESSION: CLINICAL - Prolactinomas, Thyrotropinomas (1:30 PM-3:30 PM)

Title Role of Octreotide Therapy in a Patient with TSH-Secreting Pituitary Adenoma Awaiting Surgery

Author String V Agrawal, M Zena, J Kothapally, A Drincic
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Body BACKGROUND: TSH-producing pituitary adenoma (TSH-oma) may have variable clinical presentations. Pre-treatment with somatostatin analogues helps solidify the diagnosis and restore euthyroidism while tumor removal is sought for.

CLINICAL CASE: A 34 year old healthy white male presented with the complaint of worsening dyspnea on exertion. He is physically active engaged in weight lifting exercises, and noticed worsening dyspnea with exercise for a few months. Upon review of systems, patient described mild hand tremors for years and occasional palpitations, of recent onset, lasting about a few seconds. Physical examination showed mild thyroid enlargement, warm and moist skin and fine hand tremors. As a part of initial work up, thyroid function tests (TFTs) revealed elevated TSH value of 8.81 mIU/mL (0.34-5.6), FT4 3.45 ng/dL (0.60-1.60) and FT3 9.3 pg/mL (2.3-3.9). No family history of thyroid disease was present. Repeat TFTs confirmed the initial results and subsequently a pituitary MRI was obtained which showed a 1.3 x1.7 x1.4 cm mass within the sella with mild compression of the optic chiasma and minimal extension into the cavernous sinuses bilaterally. The rest of the laboratory work-up revealed: alpha subunit 3.4 ng/ml (normal, <0.6), total T4 18.2 mcg/dl (6.1-12.2), total T3 326 ng/dl (87-178), TBG 14 mcg/ml (13-30), TSI 85% (0-129 %), prolactin 6 ng/ml (2.6-13.1), IGF-I 207 ng/ml (89-350), GH 0.68 ng/ml (0.01-1), ACTH 19 pg/ml (7-69), cortisol 11 mcg/dL , LH 3.7 mIU/ml (1.2-8.6), FSH 5 mIU/ml (1.3-19), total testosterone 1016 ng/dl (300-1080), free testosterone 85 pg/ml (47-244) and SHBG 139 nmol/L (11-80). Diagnosis of TSH-oma was established with the presence of elevated alpha subunit, elevated SHBG and MRI findings. To solidify diagnosis and in order to achieve euthyroidism, Octreotide therapy was started. A month later, repeat TFTs revealed a TSH level of 4.34 mIU/l and FT4, FT3 values of 1.75 ng/dl and 5.1 pg/ml respectively. Patient is currently awaiting pituitary tumor resection.

CONCLUSION: TSH-omas are a rare cause of thyrotoxicosis and may have dramatic consequences for the patient if left undiagnosed and untreated. Pituitary adenectomy is considered first line therapy. Medical treatment is considered adjunctive or an alternative when surgery is contraindicated or deferred. With this case, we thus propose that pretreatment with somatostatin analogues can help quickly restore euthyroidism while surgery is awaited.

Nothing to Disclose: VA, MZ, JK, AD

Pub # P3-329

Session Information POSTER SESSION: CLINICAL - Prolactinomas, Thyrotropinomas (1:30 PM-3:30 PM)

Title Differential Diagnosis of Cushing Disease from Pseudo-Cushing State by Midnight Serum Cortisol under Alprazolam Treatment

Author String S Sakihara, S Takayasu, K Terui, T Nigawara, K Kageyama, T Suda
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Body Pseudo-Cushing state (PC) is sometimes pointed out in the stressful conditions, such as depression, malnutrition, and alcoholism, in which hypothalamus-pituitary-adrenal (HPA) axis is activated. Several endocrinological data of PC are similar to those of ACTH dependent Cushing's syndrome (ACS), so that it is not easy to differentiate subjects with PC from ACS patients by the first line examinations. Measurement of midnight cortisol, either in serum or in salivary, has been valuable to diagnose ACS. This examination, however, often failed to differentiate PC from ACS. As the primary cause of PC is proposed to overactive hypothalamic CRF neuron, GABA-A receptor agonist which can suppress the neuronal excitation might calm the activated HPA axis in PC; furthermore, it might differentiate PC from ACS easily.

In this study, we examined whether alprazolam (Apz), GABA-A receptor agonist, could normalize the hypercortisolemia at midnight in PC. Midnight serum cortisol (Mn-F) was measured several times in PC (5 cases), ACS (7 cases) including 2 cases of ectopic ACTH syndrome (EAS), and healthy control (HC) (7 cases). At least once among these measurements, blood was collected under the Apz (0.4 mg) treatment, then serum cortisol concentrations were compared between with or without Apz. Without Apz treatment, Mn-F in each groups were 8.3 ± 1.7 mg/dl (PC), 19.4 ± 5.2 mg/dl (ACS), and 3.9 ± 0.3 mg/dl (HC), respectively. On the other hand, with Apz treatment, they were 4.2 ± 0.2 mg/dl (PC), 20.7 ± 5.5 mg/dl (ACS), and 3.6 ± 0.2 mg/dl (HC), respectively. Mn-F were significantly decreased by Apz treatment in PC, while they were not affected in ACS and HC. Under the treatment with Apz, Mn-F levels in PC and ACS were significantly different and could be differentiated clearly each other.

These results indicate Mn-F measurement under Apz treatment is useful in the differential diagnosis ACS from PC. Desmopressin (DDAVP) test and dexamethasone-suppressed corticotropin-releasing hormone (DEX/CRH) stimulation test are recommended examinations to differentiate ACS from PC. But, the usability and reliability of them are not fully satisfied. We suggest that Mn-F should be measured under Apz treatment at first, if PC is suspected.

Nothing to Disclose: SS, ST, KT, TN, KK, TS

Pub # P3-330

Session Information POSTER SESSION: CLINICAL - Prolactinomas, Thyrotropinomas (1:30 PM-3:30 PM)

Title Cushing Disease Discovered in a Patient with Diabetes Mellitus and Menstrual Irregularity

Author String R Chimakurthi, S Chandrasekaran, NI Steinle
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Body

Introduction: Cushing's disease constitutes 65-70 % of all cases of Cushing's syndrome. Clinical manifestations of Cushing's syndrome include metabolic abnormalities such as glucose intolerance, central obesity, dyslipidemia, cardio-vascular disease, and hypertension. Overt hyperglycemia occurs in only 10-15% of patients with the disease.

Clinical Case: A 44 year old obese Caucasian woman was referred to the diabetes center for management of her uncontrolled type 2 Diabetes Mellitus. Her diabetes was diagnosed at the age of 33 years. She also presented with fatigue, weakness, and chronic easy bruisability for which she had an extensive negative work ups performed by a dermatologist and hematologist. Her other past history included hypertension, migraine head-aches, and [ldquo]PCOS.[rdquo] Medications included aspirin, glyburide, metformin, progesterone, insulin and exenatide. Physical exam was significant for central obesity with a BMI of 31, fine facial hair, thin extremities and fragile skin. There were no bruises or striae. These signs and symptoms together with the history of bruising raised the suspicion of Cushing's syndrome. A bed-time salivary cortisol was 0.31mcg/dl (0.04-0.56). Repeat overnight 1 mg dexamethasone suppression tests revealed cortisol levels of 15.3 and 18.6 mcg/dl (4-22). 24 hour urine free cortisol was 174.9 mcg (4.0-50.0). The ACTH was found to be 33 pg/ml (5-27) with a serum cortisol level of 20.3 mcg/dl (4.0-22.0) drawn simultaneously confirming the diagnosis of ACTH dependent Cushing's disease. Subsequently, an MRI of the brain demonstrated a 9 mm pituitary microadenoma without evidence of optic nerve or optic chiasma compression. Bone density was within normal limits. The patient underwent an endonasal microscopic resection of her microadenoma. Two months post-operatively, there was no evidence of residual pituitary disease. Body weight had reduced by 40 pounds, normal menses has resumed, and all diabetes and blood pressure medications have been discontinued.

Conclusion: Cushing's syndrome should be suspected among patients with early onset type 2 diabetes, hypertension, menstrual irregularities, fragile skin and easy bruising. Both the medical history and physical exam are important in eliciting signs and symptoms of suspected Cushing's disease.

Nothing to Disclose: RC, SC, NIS

Pub # P3-331

Session Information POSTER SESSION: CLINICAL - Prolactinomas, Thyrotropinomas (1:30 PM-3:30 PM)

Title Paradoxical Results after Inadvertent Use of ACTH(1-24) Rather Than ACTHrel (Ovine Corticotropin-Releasing Hormone) during Inferior Petrosal Sinus Sampling

Author String AJH Fisco, TB Carroll, RJ Auchus, JW Findling
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Body

Introduction: Corticotropin releasing hormone (CRH) is routinely used in the evaluation of Cushing's syndrome (CS) during inferior petrosal sinus sampling (IPSS). In the USA, ovine CRH (oCRH) is marketed as ACTHrel, a name that could easily be confused with the pituitary hormone that it stimulates, ACTH. This report details the inadvertent use of ACTH(1-24) (cosyntropin) during IPSS in two different centers and the confounding results obtained.

Clinical Cases: The two patients are a 28 yo F and a 34 yo F who presented with unequivocal evidence of endogenous ACTH-dependent CS. MRI of the sella in both cases showed no convincing pituitary abnormalities. Both patients underwent IPSS to confirm the presence or absence of pituitary ACTH dependent CS [Cushing's disease (CD)].

Patient #1 had pre-infusion ACTH values of 3,780 pg/mL [Right inferior petrosal sinus (IPS)], 106 pg/mL (Left IPS), and 22 pg/mL [peripheral (P)] (normal P 5-46 pg/mL) with post infusion ACTH nadir values of 130 pg/mL (Right IPS), 48 pg/mL (Left IPS), and 26 pg/mL (P).

Patient #2 had pre-infusion ACTH values of 30 pg/mL (Right IPS), 70 pg/mL (Left IPS), and 21 pg/mL (P) with post infusion ACTH nadir values of 2 pg/mL (Right IPS), 6 pg/mL (Left IPS), and 2 pg/mL (P). The IPSS results triggered re-evaluation of the procedures, which discovered that ACTH(1-24) was given instead of oCRH (ACTHrel) in both patients. The high basal IPS:P ACTH ratios indicated CD, and both patients had surgical proven CD.

Discussion: Both ACTH(1-24) and oCRH (ACTHrel) are used for dynamic testing of the hypothalamic-pituitary-adrenal axis. Current [sandwich] immunometric assays measure ACTH(1-39). The decrease in plasma ACTH concentration after ACTH(1-24) infusion is an *in vitro* artifact. The ACTH(1-24) binds only one antibody effectively and competes with endogenous ACTH(1-39) for sandwich complex formation. This competition causes a spurious decrease in measured plasma ACTH (1). This finding is especially important, since some patients with pituitary ACTH-dependent CS may only have a pituitary ACTH gradient during IPSS after CRH infusion, and the use of ACTH(1-24) may lead to an incorrect diagnosis of ectopic ACTH secretion.

Conclusion: Labeling of oCRH as ACTHrel may cause confusion with ACTH(1-24). A paradoxical decrease in plasma ACTH after presumed oCRH infusion should suggest the inadvertent use of ACTH(1-24).

(1) Raff H, Findling J, Wong J. Short Loop Adrenocorticotropin (ACTH) Feedback after ACTH-(1-24) Injection in Man Is an Artifact of the Immunoradiometric Assay. *Journal of Clinical Endocrinology and Metabolism*. 1989; 69:678-80.

Disclosures: TBC: Investigator, Corcept Therapeutics. RJA: Investigator, Corcept Therapeutics; Novartis Pharmaceuticals. JWF: Consultant, Corcept Therapeutics; Novartis Pharmaceuticals; Investigator, Corcept Therapeutics; Novartis Pharmaceuticals. Nothing to Disclose: AJHF

Pub # P3-332

Session Information POSTER SESSION: CLINICAL - Prolactinomas, Thyrotropinomas (1:30 PM-3:30 PM)

Title Zoledronic Acid and Acute Renal Failure in a Patient with Cushing Disease

Author String MA Czepielewski, A Boschi, F Costenaro
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Body

Hypercortisolism has many systemic manifestations including severe bone mass loss. Osteoporosis has been recognized as a frequent morbid condition even at mild cases of this syndrome. Recently, zoledronic acid (ZA) has been approved by FDA as an adjunctive drug on treatment of chronic glucocorticoid use induced osteoporosis. Indeed, ZA was included in the management of osteoporosis for Cushing[acute]s Disease (CD). Our objective is to describe one girl with confirmed CD and symptomatic osteoporosis, who suffered acute nephropathy soon after ZA infusion that was observed at our Endocrinology Division.

A 22 years old female came to our clinic complaining about weight gain, hypertension and weakness. During evaluation, we found an ACTH dependent CD. After sinus petrosal catheterization confirmed pituitary origin of disease, we proceeded to transsphenoidal surgery. Unfortunately, she was not cured by neurosurgery so we went to treatment with ketoconazole plus central nervous system radiotherapy. One year after that, she was treated with ketoconazole 300 mg/day, but still symptomatic of CD, when we found 3 costal arch fractures associated with low bone mass density (T -4,1 at vertebrae), so we started to treat her with calcium and vitamin D supplementation. Later on, we prescribed a shot of zoledronic acid, but after 4 days she showed a skin rash, creatinine increase and symptomatic hyperkalemia, reverted after a week of management without dialysis. This adverse reaction occurred during treatment with, captopril 75 mg/day, espironolactone 25 mg/day and atenolol 100 mg/day for hypertension, although she had no previous kidney impairment or drug related nephron injury.

In November 2009, FDA reported a series of 24 patients that suffered acute renal failure, including 7 deaths, associated with ZA infusion for the treatment osteoporosis. At that time, FDA recommended against ZA acid use in patients with renal function impairment and other co-morbidities. Our observation suggests Cushing disease patients, who usually have many severe co-morbidities related to endogenous hypercortisolism, might be a group in greater risk for kidney failure related to ZA use and should be monitored.

Nothing to Disclose: MAC, AB, FC

Pub # P3-333

Session Information POSTER SESSION: CLINICAL - Prolactinomas, Thyrotropinomas (1:30 PM-3:30 PM)

Title Silent Corticotroph Pituitary Adenomas -- The Mississippi Experience

Author String AW Gannon, N Chaithongdi, AD Parent, J Fratkin, E Melcescu, AA Galhom, WC Nicholas, CA Koch
University of Mississippi, Jackson, MS; University of Mississippi, Jackson, MS; University of Mississippi,
Jackson, MS; Suez Canal University, Ismailia, Egypt

Body Background: Silent corticotroph adenomas (SCAs) are pituitary tumors with positive immunohistochemical staining for ACTH but without clinical evidence of excess cortisol. SCAs have the potential to progress to overt Cushing's disease, therefore close monitoring is necessary (1,2).
Methods: We retrospectively reviewed our hospital's pituitary database from 1979 to 2010, and identified 740 cases, 12 of which (1.6%) were histopathologically SCAs. One of those 12 patients had a 10 year follow-up.
Clinical case: A 52 year-old black woman presented to the ENT clinic due to nasal congestion and was found to have a sphenoid sinus mass. MRI demonstrated an enlarged sella with normal appearing pituitary gland and exophytic pituitary adenoma growing into the sphenoid sinus. No clinical features of Cushing's syndrome (CS) were present. Hormone workup: ACTH 168 pg/mL (10-60), cortisol 13.3 mcg/dL at 1500 and 15.7 mcg/dL at 0600, 24 h UFC 45.3 mcg (2.1-38), remainder normal. The patient underwent transsphenoidal resection (TSS). Pathology revealed pituitary corticotroph adenoma. Initially, she had no clinical issues. Followup imaging demonstrated residual tumor. Over the next few years she slowly developed overt CS. MR demonstrated pituitary adenoma enlargement and her 24 h UFC increased remarkably to 1862 mcg. She underwent repeat TSS and postoperative radiotherapy with some success. Seven years later she presented with significant mass recurrence and CN III palsy. She subsequently received a third TSS and now has an unresectable tumor. She continues to show biochemical evidence of cortisol excess.
Conclusion: SCAs are known to behave more aggressively than other non-functioning adenomas. Predicting their behavior is challenging (1,2). SCAs usually present with macroadenoma as opposed to CD which mostly presents with smaller tumors (2). Resection of these tumors can thus be more challenging. The Cushing's phenotype is usually not shown in these patients due to abnormal ACTH structure, diminished adrenal gland responsiveness or simply lower levels of ACTH expression. For reasons that are unclear, SCAs have a potential for evolving into CD and it is not currently possible to predict in which patient this evolution will occur. Likely, underlying yet undiscovered or unrecognized genetic predispositions are the cause and may be triggered by radiation. In our database, only one patient continued to followup longterm, making it difficult to estimate recurrence rates.

(1) Erickson D et al., Clin Endocrinol 2009; 71:92-99.
(2) Cooper O et al., Horm Cancer 2010; 1 (2): 80-92.

Nothing to Disclose: AWG, NC, ADP, JF, EM, AAG, WCN, CAK

Pub # P3-334

Session Information POSTER SESSION: CLINICAL - Prolactinomas, Thyrotropinomas (1:30 PM-3:30 PM)

Title ACTH-Secreting Adenomas: Size Does Not Correlate with Hormonal Activity

Author String N Mathioudakis, C Pendleton, A Quinones-Hinojosa, R Salvatori
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Body **INTRODUCTION:** Cushing's disease (CD) due to macroadenomas (MCAs) is uncommon. While these tumors have been reported to have higher plasma ACTH and serum cortisol levels than microadenomas (mCAs), it is unclear whether MCAs result in more overt clinical manifestations of hypercortisolemia. **METHODS:** We performed a retrospective review of 33 newly diagnosed CD patients (12 MCAs) seen by a single endocrinologist in an academic center over a 10-year period. We quantified the number of signs and symptoms of hypercortisolism and of mass effect documented in the initial visit, and arbitrarily defined as "mild" cases with less than 30% of the clinical manifestations. **RESULTS:** We found that 4/21 (19%) of the mCAs and 9/12 (75%) of the MCAs had mild hypercortisolism. Comparing mild MCAs with all the mCAs, we found that mean age was 39.9±4.5 in mild MCAs and 33.5±2.6 yrs in mCAs, % females was 78 and 95, BMI 39.6±2.2 and 32.8±1.5 kg/m², blood pressure 139/86 and 137/84 mmHg, tumor diameter 1.91±0.27 and 0.46±0.04 cm. Looking at mass effect, 56% of the mild MCAs had optic chiasm compression, 44% cavernous sinus invasion, and 11% cranial nerve palsy. In the mild MCAs, 28.6±3.1% of signs, 20.9 ±1.8% of symptoms, and overall 23.9±1.3% of clinical manifestations (signs/symptoms) of hypercortisolism were present. In the mCA group, 38.9±2.9% of signs, 32.0±2.2% of symptoms, and overall 34.7±2.1% of clinical manifestations were present. While obesity, facial fullness, dorsocervical fat, and hypertension occurred in similar frequency in the two groups, most of the clinical features deemed to have the highest CD discriminatory capacity (easy bruising, facial plethora, proximal muscle weakness), were less frequent in mild MCAs. In the mild MCAs, the mean ACTH and cortisol were 84.5±14.5 pg/ml and 469.7±97.7 nmol/L respectively, with a mean cortisol:ACTH ratio of 6.1±1.3; in the mCAs, the ACTH and cortisol were 64.6±7.3 pg/ml and 652.7±58.4 nmol/L, corresponding to a ratio of 12.8±1.9. **CONCLUSIONS:** The size of an ACTH-secreting pituitary adenoma does not necessarily correlate with the extent of clinical manifestations of hypercortisolism. In fact, these variables may be inversely related. The difference in cortisol:ACTH ratio suggests that diminished biological activity of ACTH and/or immature corticotroph cell differentiation in mild MCAs may account for the phenomenon of [ldquo]quiet[rdquo] hypercortisolemia in these adenomas (1).

Raverot G, Wierinckx A, Jouanneau E, et al. Clinical, hormonal and molecular characterization of pituitary ACTH adenomas without (silent corticotroph adenomas) and with Cushing's disease. *Eur J Endocrinol*;163 (1):35-43.

Sources of Research Support: Dr. Mathioudakis was supported in part by NIH T32 training grant.

Nothing to Disclose: NM, CP, AQ-H, RS

Pub # P3-335

Session Information POSTER SESSION: CLINICAL - Prolactinomas, Thyrotropinomas (1:30 PM-3:30 PM)

Title Diagnostic Data and Post-Surgical Follow-up in Cushing Disease Patients Due to Pituitary Macroadenomas

Author String AEE Alcantara, ACL Pereira, NRC Musolino, VAS Cascato, MD Bronstein, MCBV Fragoso, MC Machado University of São Paulo Medical School, São Paulo, Brazil

Body

Background: Pituitary macroadenomas (≥ 10 mm) represent 10 to 20% of Cushing's disease (CD) patients. Previous studies suggest some different laboratorial data such as lower cortisol suppression after high dose of dexamethasone test, less response do CRH and tendency to lower post surgical remission rate. However, there are few publications about this patient's subgroup.

Objective: To report laboratorial data, MRI, remission rate and post surgical follow up in group of CD patients due to pituitary macroadenomas.

Material and Methods: Between 1990 and 2010, 48 CD patients with macroadenomas were evaluated, 85% female patients, 32 yrs-old (9-70). The diagnosis of ACTH-dependent Cushing's syndrome (ADCS) was established by elevated urinary total cortisol (UTC) levels, no cortisol (Fs) suppression after low dose dexamethasone test, loss of circadian rhythm of cortisol secretion and elevated or inappropriate ACTH levels. All of them were submitted to sellar MRI e were defined as macroadenomas when highest diameter of lesion was ≥ 10 mm. The diagnosis of CD was confirmed after pathological analysis showing pituitary adenoma with positive ACTH staining (n=42, 87.5%). Mean follow-up was 63.5 months (6-180).

Results: At diagnosis, mean UTC of each patient was 841.3 ± 730.2 [μ]g/24h (reference: 30-300), however, 12% of them presented one or more normal samples. In MRI, the maximum diameter was 16 ± 7.2 mm (10-45) 8 cases with cavernous sinus (CS) invasion and one case (45 mm) with third ventricle and anterior fosse extension. 77% (24/31) responded to desmopressin test and 82.4% (14/17) showed cortisol suppression after overnight 8 mg dexamethasone test ($>50\%$). Overall post surgical remission rate was 63% (29/46), but higher remission (85.7%, 12/14) occurred in subgroup of patients with smaller tumors (10-13 mm, 2 with CS invasion) as compared to those with large tumors (14-45 mm, 7 with some invasion): 43.8% (7/16). Recurrence occurred in 20.7% of cases (6/29), after 74.5 ± 33.2 months of follow up (15-108).

Conclusion: Although a comparative CD group with microadenomas was not analyzed concurrently in this study, the laboratorial data, dynamic tests and post surgical outcome in these patients with CD due to pituitary macroadenomas appeared to be similar to those with microadenomas. However, this is a heterogeneous subgroup of patients as smaller macroadenomas presented better post surgical outcome than those with larger macroadenomas.

Nothing to Disclose: AEEA, ACLP, NRCM, VASC, MDB, MCBVF, MCM

Pub # P3-336
Session Information POSTER SESSION: CLINICAL - Prolactinomas, Thyrotropinomas (1:30 PM-3:30 PM)
Title Hypercortisolism Control with Cabergoline First-Line Long-Term Monotherapy in Cushing Disease Due to an Invasive Macroadenoma
Author String LT Moura, MC Machado, MC Fragoso, MD Bronstein
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Body **Background:** Cushing's disease (CD) as a result of a pituitary macroadenoma is an uncommon etiology of Cushing's syndrome (CS). Cabergoline (CBG), a long-acting dopamine subtype 2 receptor agonist, has been used in limited cases of Nelson's syndrome (NS), ectopic ACTH-secreting tumors and recently in CD patients. Few studies have shown that long term therapy with CBG normalized urinary free cortisol (UFC) in CD cases not controlled by surgery. Furthermore, it has been demonstrated ACTH reduction and inducing tumor shrinkage in cases of NS. **Objective:** To evaluate the long-term efficacy of CBG monotherapy in CD patient due to an invasive macroadenoma. **Case report:** A 42 year woman with amenorrhea in the last 6 months, weight gain (6 Kg), proximal muscle weakness and easy bruising. At admission, although she presented normal BMI (22,8 kg/m²), she had facial plethora, abdominal and trunked fat distribution and thinner skin. ACTH-dependent CS was confirmed by elevated UFC, no suppression of cortisol after low dose of dexamethasone, upper limit of salivary midnight cortisol and elevated ACTH. MRI revealed a sellar mass of 1.7 cm of maximum diameter with extension to the left cavernous sinus and right deviation of the stalk. The surgical and radiotherapy treatment were refused by the patient at that moment. CBG was initiated at 0.5 mg 3 times a week. After 6 months of regular CBG use without side effects, she had complete clinical and laboratorial remission, although the maintaining of tumor size. In attempt to optimizing tumoral size reduction, CBG was increased to 0.5 mg per day. 9 months after, new MRI revealed a 0.5 cm increase of tumor size instead of complete clinical and laboratorial control. After 2 yrs in continue use of CBG she improved her metabolic syndrome and maintain normal UFC. Nowadays she accepted be submit to surgery and/or radiotherapy to control the tumor size progression. **Discussion and Conclusion:** We used CBG as first-line therapy in a patient with invasive macroadenoma with complete clinical and laboratorial response. This case demonstrated that although the hormonal production was controlled, the molecular mechanism involved in the tumor progress could not be controlled with CBG. There are very limited cases treated with CBG as first-line monotherapy in CD due to macroadenoma. Larger number of patients will be required to establish a possible relationship between degree of hypercortisolism, CBG dosage, and response to tumor size control.

- (1) Godbout A, Manavela M, Danilowicz K, Beauregard H, Bruno OD, Lacroix A. Cabergoline monotherapy in the long-term treatment of Cushing's disease. *European Journal of Endocrinology* (2010) 163:709-716.
- (2) Vilar L, Naves LA, Azevedo MF, Arruda MJ, Arahata CM, Silva LM, Agra R, Pontes L, Montenegro L, Albuquerque JL, Canadas V. Effectiveness of cabergoline in monotherapy and combined with ketoconazole in the management of Cushing's disease. *Pituitary* (2010) 13: 123-129.

Nothing to Disclose: LTM, MCM, MCF, MDB

Pub # P3-337

Session Information POSTER SESSION: CLINICAL - Prolactinomas, Thyrotropinomas (1:30 PM-3:30 PM)

Title Preoperative and Long-Term Postoperative Ketoconazole Treatment in Cushing Disease: Clinical Aspects and Plasma ACTH Behavior during Its Use

Author String SR Correa-Silva, P Gaeta, GM Alves, MRA Martins, J Abucham, A-MJ Lengyel
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Body Transphenoidal surgery (TS) is the treatment of choice in Cushing's disease (CD), but it can be inefficient in about 20% of the microadenomas and 60% of the macroadenomas. Radiotherapy and adrenalectomy have their limitations. Ketoconazole is a steroidogenesis inhibitor which has been used for the treatment of CD for many years, usually for short periods of time. There are few studies with large number of patients with CD treated during long-term with ketoconazole. Normalization of urinary free cortisol (UFC) was seen in 50 to 93% of the patients. The effect ketoconazole use on ACTH secretion in CD is controversial.

In our study 32 patients with CD received ketoconazole, of whom 25 preoperatively and 16 postoperatively. The initial dose was 300-400mg/day, which was then titrated accordingly to UFC levels, to the maximum of 300-1500mg/d. Mean follow-up time was 5.6 months (1-12) in the preoperative group and 31.5 (1-81) in the postoperative. UFC levels, which were evaluated as percentage of the upper limit of normality (% ULN), decreased significantly in both groups during treatment (preoperative: before: 631 ± 135 %ULN; last evaluation: 122 ± 29.2 and postoperative: before: 220 ± 58.4 ; last evaluation: 146 ± 69.6). In the preoperative and postoperative groups there were reduction in UFC values in 96% and 81% of the patients and normalization in 65% and 63%, respectively. There was improvement in weight (58%), muscular weakness (86%), hypertension (67%) and diabetes mellitus (88%) preoperatively. Plasma ACTH levels were evaluated in patients that received ketoconazole before surgery. There was a significant increase in ACTH values during treatment (before: 62 ± 7 pg/mL; last evaluation: 92 ± 15.2). In the 12 patients who had serial ACTH evaluation (1stweek: 64.7 ± 9.6 ; 1stmonth: 64.2 ± 7.8 ; 3rdmonth: 90.6 ± 17.9 ; 6thmonth: 98.2 ± 20), a significant increase in ACTH values occurred only at the 6th month. Liver enzymes increased in 20% of patients, and this was usually mild and transitory.

In conclusion, ketoconazole is an efficient and safe option to treat patients with CD prior to surgery, as most of them had important clinical and biochemical improvement. Postoperatively, it was an useful tool in the long-term control of the hypercortisolism. Interestingly, a significant rise in ACTH levels was observed during the treatment. This could have occurred because of the activation of normal corticotrophs, which were earlier suppressed by hypercortisolism.

Nothing to Disclose: SRC-S, PG, GMA, MRAM, JA, A-MJL

Pub # P3-338

Session Information POSTER SESSION: CLINICAL - Prolactinomas, Thyrotropinomas (1:30 PM-3:30 PM)

Title Adrenal Crisis Abruptly Induced by Low-Dose Ketoconazole Therapy in a Patient with Cushing Disease

Author String MD Vanderlinde-Wood, DM Wynn, A Gosmanova
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Body Background: Ketoconazole therapy for hypercortisolism can cause adrenal insufficiency as reported in the literature, but on rare occasions can induce acute adrenal crisis.
Clinical Case: A previously healthy 50 year old man started showing subtle symptoms consistent with Cushing's syndrome including recurrent oral thrush, easy bruising, irritability, confusion, and muscle wasting. Signs of increased diplopia, drooping eyelids, numbness and weakness of left face in V1 and V2 nerve distribution along with diagnosis of left 3rd, 4th, and 5th cranial nerve palsy by ophthalmology led to MRI of brain which revealed a pituitary macroadenoma measuring 2.6x2.6x1.8 cm. Other labs include ACTH of 348 pg/mL (nml 0-46 pg/mL), cortisol of 59.8 mcg/dL (nml 6.0-30.0 mcg/dL), and 24 hour urinary free cortisol of 3,431 ug/24hr (nml 0-50 ug/24hr). Neurosurgery planned for trans-sphenoidal resection of the macroadenoma however, patient was found to have lung masses diagnosed as a nocardiosis infection which further delayed surgical intervention. His care was further complicated by central diabetes insipidus, central hypothyroidism, hypogonadotropic hypogonadism, and vitamin D deficiency.
Medical management was initiated for hypercortisolism with ketoconazole. He received a total of four doses of oral ketoconazole (200 mg), but after a few days, he began having severe hypotension, hyperkalemia, and hypoglycemia. He was transferred to the intensive care unit and found to have serum cortisol level down to 11.5 mcg/dL in contrast to his prior level of 59.8 mcg/dL. In the ICU, he required multiple fluid boluses, D-30 infusion for persistent hypoglycemia, and potassium-binder therapy for hyperkalemia. He was also treated with stress-doses of IV hydrocortisone with improvement of his clinical parameters. He was tapered down to maintenance dose of hydrocortisone and restarted on low dose ketoconazole. Repeat 24 hour urinary free cortisol levels dropped from 3431 ug/24hr to 406 ug/24hr within one week.
Conclusion: The goal of medical therapy for hypercortisolism in Cushing's Disease with ketoconazole is to inhibit steroidogenesis effectively in order to eventually decrease steroid levels back to acceptable levels. Rarely, however, the treatment can cause adrenal insufficiency. This case demonstrates that during treatment with ketoconazole for hypercortisolism, patients need to be monitored closely for abrupt adrenal insufficiency which may lead to adrenal crisis.

Nothing to Disclose: MDV-W, DMW, AG

Pub # P3-339

Session Information POSTER SESSION: CLINICAL - Prolactinomas, Thyrotropinomas (1:30 PM-3:30 PM)

Title Effect of Long-Acting Repeatable Octreotide on the ACTH Secretion in Nelson Syndrome and Cushing Disease

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Body Background: Management of persistent ACTH excess in Nelson[acute]s syndrome (NS) and Cushing[acute]s disease (CD) remains a challenge. Somatostatin (SS) and its analogs as octreotide decrease ACTH secretion through SS receptors (sst) of pituitary cells. To our knowledge there are no reports on the effect of long-acting repeatable octreotide (oct-lar) in patients with NS and CD who failed conventional therapy. Clinical cases: We studied one woman (*AS*) with NS and two (*LC and GG*) with persistent CD. They were all treated with oct-lar (20 mg/monthly i.m.). *AS* had received radiotherapy twice and underwent trans-cranial pituitary surgery in four different occasions. The pituitary tumor continued extending to the sphenoid sinus. She was on hydrocortisone and fludrocortisone persisting pigmented with left eye blindness and palsy of the third cranial nerve. Monthly oct-lar was prescribed; three months later ACTH levels fell from 1069.0 ± 10 pg/ml (normal [le] 50.0 pg/ml, IRMA) to 525.0 ± 13.0 pg/ml and to 321.0 ± 4.0 pg/ml after 21 months; $p=0.001$. Pigmentation decreased, visual field remained unchanged with no evidence of tumor growth. In order to determine whether previous radiotherapy was involved in the control of NS oct-lar was discontinued and ACTH increased significantly (1210.0 ± 5.0 pg/ml) $p=0.0001$, suggesting the inhibitory role of this analog. *LC* had remission of CD after selective pituitary adenectomy relapsing after 5 years. She underwent total pituitary surgery remaining with cortisol excess (UFC = 160.0 ± 20.0 [micro]g/day; normal [le] 90.0 [micro]g/day; ACTH = 59.0 ± 4.0 pg/ml) developing toxicity while on ketoconazole. After 4 monthly injections of oct-lar, UFC and ACTH levels did not show differences from baseline (130.0 ± 10.0 [micro]g/day and 66.0 ± 17.0 pg/ml, respectively); $p \geq 0.090$ for both. *GG* persisted with CS after total hypophysectomy and gamma knife radiosurgery. After a month trial with oct-lar UFC 236.0 ± 15.0 [micro]g/day and ACTH 85.0 ± 13.0 pg/ml did not differ from baseline (211.0 ± 13.8 [micro]g/day and 83.0 ± 8.0 pg/ml; respectively); $p \geq 0.767$ and clinical changes were not observed. Conclusion: Oct-lar therapy reduced ACTH secretion in NS without side effects but failed to control ACTH in CD, probably due to the inhibitory effect of glucocorticoids on sst₂ receptor expression. Further trials in CD patients should consider the combination of oct-lar with adrenostatic drugs.

Nothing to Disclose: ALA, EMC, GT, LNC

Pub # P3-340
Session Information POSTER SESSION: CLINICAL - Prolactinomas, Thyrotropinomas (1:30 PM-3:30 PM)
Title Coagulation and Fibrinolysis Parameters before and Three Months after Successful Medical Treatment for Cushing Disease
Author String R van der Pas, C de Bruin, FWG Leebeek, MPM de Maat, DC Rijken, AM Pereira, JA Romijn, RT Netea-Maier, AR Hermus, PMJ Zelissen, FH de Jong, A-J van der Lelij, WW de Herder, SWJ Lamberts, LJ Hofland, RA Feelders
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Body **Introduction**
Cushing's disease (CD) is associated with a hypercoagulable state and an increased risk for venous thrombosis. Transsphenoidal surgery is the primary treatment for CD, but the long-term remission rate is disappointing. We performed a prospective trial in which a stepwise medical treatment for CD was applied with the universal somatostatin-analog pasireotide, the dopamine agonist cabergoline and the adrenal-blocking agent ketoconazole. We now report the effect of this regimen on coagulation and fibrinolysis parameters.

Methods

17 patients with CD were treated for 80 days with pasireotide monotherapy or combination therapy with cabergoline after day 28 and, if necessary, ketoconazole after day 60. Parameters of coagulation were measured in patients with CD at baseline, day 28 and day 80 and compared to healthy controls. Moreover, clot lysis time (CLT), an in-house assay used to measure fibrinolysis, was examined. High levels of CLT have previously been shown to be positively correlated with the risk for thromboembolic events.

Results

At baseline, patients had significantly shortened activated partial thromboplastin time (ApTT) ($p=0.01$) and higher levels of fibrinogen ($p=0.04$) and protein S activity ($p=0.02$) compared to controls. The levels of von Willebrand Factor Antigen (vWF Ag) and Factor VIII:C tended to be higher in patients than in controls ($p=0.06$ and $p=0.08$, respectively). Moreover, CLT was prolonged in patients compared to controls (122.7 vs 83.9 minutes; $p=0.02$). After 80 days of treatment, 15/17 (88%) patients had normalized urinary free cortisol (UFC) levels. Whereas a slight decrease of antithrombin levels was found after treatment ($p=0.01$), no significant changes were observed for ApTT, fibrinogen, D-dimer levels, Factor VIII:C, vWF Ag, Protein C and S activity or CLT.

Discussion

Our data indicate that the hypercoagulable state in CD results from both increased production of procoagulant factors and impaired fibrinolytic capacity. Stepwise medical therapy with pasireotide, cabergoline and ketoconazole normalizes UFC levels in 88% of patients within 3 months. This biochemical remission is not accompanied by major changes in procoagulant factors and fibrinolysis. This suggests that sustained control of hypercortisolism is required to reverse the hypercoagulable state in CD which may have implications for the duration of thromboprophylaxis in patients with (cured) CD.

Sources of Research Support: Investigator initiated study sponsored by Novartis.

Disclosures: WWdH: Advisory Group Member, Novartis Pharmaceuticals; Ipsen. Nothing to Disclose: RvdP, CdB, FWGL, MPMdM, DCR, AMP, JAR, RTN-M, ARH, PMJZ, FHd, A-JvdL, SWJL, LJH, RAF

Pub # P3-341

Session Information POSTER SESSION: CLINICAL - Prolactinomas, Thyrotropinomas (1:30 PM-3:30 PM)

Title Changes in Appetite and PYY Levels in Cushing Disease before and after Transsphenoidal Surgery

Author String EB Geer, IM Conwell, KD Post, PU Freda
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Body

Background: Chronic glucocorticoid excess due to Cushing's Disease (CD) is associated with increased appetite. The gut hormone PYY is released during meals and acts centrally to increase satiety.

Objective: To examine changes in PYY, and appetite scores, measured by visual analogue scale (VAS), in CD before and after surgical treatment in an attempt to understand the mechanisms of increased appetite in CD and its change with cure.

Methods: 12 female patients with newly diagnosed CD, mean age 36 yr (range 23-64) and mean weight 86.4 ± 21.9 kg, were studied pre-op (v1) and twice post-operatively in remission: after 6 mos. on a stable dose of physiologic oral glucocorticoids (GCs) (v2, n= 9) then again 6 mos. after GCs had been discontinued (v3, n=9). Each visit included measurement of fasting and post-test meal (over 3 hrs, Optifast, Novartis) total PYY. Hunger was measured by VAS and PYY was measured by ELISA (Linco). Data are presented as mean \pm SD and differences were assessed by two-tailed paired t-test.

Results: Peak post-meal PYY was higher at v3 vs. v2 (v3: 144.3 ± 56.0 pg/ml vs. v2: 108.2 ± 48.4 , $p=0.05$) but was not different than v1 (147.0 ± 38.7 , $p=0.8$ vs. v3, $p=0.08$ vs. v2). PYY peaked earlier at v3 vs. v2 (v3: 66.7 min vs. v2: 120 min, $p = 0.005$). PYY area under curve (AUC) increased significantly from v2 to v3 (v2: 290.2 ± 144.1 pg.hr/ml vs. v3: 331.9 ± 121.4 , $p = 0.045$). Post-meal hunger decreased from v1 to v2 ($p=0.04$). Pre- and post-meal fullness did not change pre- to post-op. Pre-meal satisfaction increased from v1 to v3 ($p=0.06$) and pre-meal prospective consumption decreased from v1 to v2 ($p=0.01$) and v1 to v3 ($p=0.03$). Weight significantly decreased from v1 to v2, v2 to v3, and v1 to v3 ($p=0.008$, 0.02 , and 0.008).

Conclusions: Despite decreased endogenous cortisol, post-operative CD patients in remission showed a trend towards decreased peak PYY values while taking exogenous glucocorticoids. After oral GCs were discontinued, peak post-meal PYY and AUC significantly increased, and time to peak significantly decreased. Some measures of satiety and desire to eat changed with remission. The effects of endogenous vs. exogenous glucocorticoids on appetite and appetite hormones need further investigation in larger numbers of patients.

Sources of Research Support: NIH Grant K23 DK 082617 (to EBG), K24 DK 073040 (to PUF), CReFF award (to EBG), Mount Sinai General Clinical Research Center MO1-RR-00071 and CTSA grant UL1RR029887.

Nothing to Disclose: EBG, IMC, KDP, PUF

Pub # P3-342

Session Information POSTER SESSION: CLINICAL - Prolactinomas, Thyrotropinomas (1:30 PM-3:30 PM)

Title Course of Pregnancy in Cushing Disease under Different Management Approaches (No Treatment, Surgery, Gamma-Knife or Ketoconazole)

Author String S Cannavo, M Losa, OR Cotta, L Curto, F Ferrau, M Ragonese, ML Torre, F Trimarchi
University of Messina, Messina, Italy; San Raffaele Hospital, Milano, Italy

Body **Background:** Pregnancy in patients with Cushing's disease (CD) is rare and considered at risk for mother and fetus. Until now, very few data concerning the consequences of mild hypercortisolism or the effects of different treatment options on the outcome of gestation are available (1, 2).
Objective: We present the course of 5 pregnancies in 3 women with mild CD.
Patients: The first patient, in whom hypercortisolism was diagnosed during the first trimester of pregnancy, was untreated, the second one was treated only with gamma-knife (GK), and the last one (who became pregnant 3 times in few years) had been previously operated and treated with GK and ketoconazole.
Results: In the first two patients, pregnancies were uneventful and full-term. Central hypothyroidism was diagnosed in both patients, during the second or the third trimester of pregnancy. Elective caesarian section was planned only in the second patient. The newborns were healthy and normal-weight, and grew appropriately thereafter. In the first patient, DDAVP and h-CRH tests were performed during the first trimester of gestation and after delivery. ACTH increases after h-CRH and DDAVP administration were higher than in age-matched healthy non pregnant women, but h-CRH-stimulated ACTH levels were significantly higher after delivery than during pregnancy. In the third woman, two pregnancies occurring 3 years after GK and few months after ketoconazole withdrawal were interrupted by spontaneous abortion or placental disruption, despite normal cortisol levels. This patient became again pregnant 3 years later and delivered vaginally a healthy full-term infant. Agalactia and low serum PRL levels occurred only in this case.
Conclusions: Pregnancies occurring in patients treated with GK should be considered at risk, despite normalization of cortisol levels. h-CRH and DDAVP tests are useful and safe tools for the diagnosis of CD also in pregnant women. Pregnant women with CD are at increased risk for hypothyroidism. Low PRL levels and agalactia can occur in patients treated by GK.

- 1) Lindsay JR et al., *Endocr Rev* 2005; 26:775
- 2) Chiodini I et al., *J Endocrinol Invest* 2004; 27:954

Nothing to Disclose: SC, ML, ORC, LC, FF, MR, MLT, FT

Pub # P3-343

Session Information POSTER SESSION: CLINICAL - Prolactinomas, Thyrotropinomas (1:30 PM-3:30 PM)

Title Illness Perceptions, Quality of Life and Self-Reported Physical Symptoms in Patients after Long-Term Remission of Cushing Syndrome

Author String J Tiemensma, AA Kaptein, AM Pereira, JWA Smit, JA Romijn, NR Biermasz
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Body

Context and objective: Illness perceptions pertain the pattern of beliefs patients develop about their illness. These views are determinants of behavior directed at the illness. Illness perceptions are determinants of quality of life (QoL). QoL remains impaired in patients after treatment of endogenous hypercortisolism, but illness perceptions were never studied in these patients. The objective of the current study was to explore illness perceptions and its relation with QoL in patients treated for Cushing's syndrome (CS).

Design: This was a cross sectional study.

Subjects: We included patients in long-term remission of Cushing's syndrome (n=52), and compared them with five reference populations: patients with vestibular schwannoma (n=80), head and neck cancer (n=68), acute (n=35) or chronic (n=63) pain, and COPD (n=171). Illness perceptions were evaluated using the Illness Perception Questionnaire-Revised. The protocol was approved by the Medical Ethics Committee.

Results: Patients after long-term remission of CS reported more negative illness perceptions compared to reference groups. Patients with CS scored distinctively worse compared to patients with vestibular schwannoma, patients with head and neck cancer, and patients with acute pain, and also reported more illness related complaints. There were also some differences in illness perceptions between patients with CS and patients with chronic pain en patients with COPD, but there was no distinct pattern. Illness perceptions showed a strong correlation with QoL.

Conclusion: Patients after long-term remission of Cushing's syndrome report more negative illness perceptions compared to patients with other acute or chronic conditions. The results strongly point towards the need for a self-management intervention aimed to improve these illness perceptions, and thereby QoL.

Nothing to Disclose: JT, AAK, AMP, JWAS, JAR, NRB

Pub # P3-344

Session Information POSTER SESSION: CLINICAL - Prolactinomas, Thyrotropinomas (1:30 PM-3:30 PM)

Title Significant MRI-Measured Body Composition Changes in Female Patients with Cushing Disease after Transsphenoidal Surgery

Author String EB Geer, W Shen, KD Post, PU Freda
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Body **Context:** Cushing's Disease (CD) results in abnormal adipose tissue (AT) distribution. Remission from active disease may reverse these changes.
Objectives: To use whole-body magnetic resonance imaging (MRI) to examine lean and AT distribution in female patients with CD before and over time after transsphenoidal surgery (TS)-induced remission, to further understand the role of glucocorticoid excess in the development of abnormal AT distribution and obesity.
Design, Setting, and Patients: A prospective study in 10 female patients with CD who were studied before TS, 6 months post-op, and again 6 months after discontinuing glucocorticoids.
Outcome Measures: Mass of skeletal muscle (SM) and AT in the visceral (VAT), subcutaneous (SAT), and inter-muscular (IMAT) compartments from whole-body MRI and serum levels of insulin, glucose, leptin, and high molecular weight adiponectin (HMWApM) were measured before and up to 3.5 years after surgery.
Results: Weight, Total AT (TAT), VAT, SAT, trunk SAT (TrSAT), limb SAT, and SM decreased significantly following surgery ($p < 0.05$). Mean HOMA-IR and leptin decreased significantly ($p < 0.01$), but HMWApN did not significantly change. Other than TrSAT/SAT, AT ratios did not significantly change.
Conclusions: Up to 3.5 years after TS for CD, there were significant decreases in weight and masses of all AT depots except IMAT. However, SM continued to decline, and AT distribution generally did not change. These findings have implications for understanding of the role of glucocorticoids in the abnormal AT distribution seen in patients exposed to chronic excess glucocorticoids, and the body composition changes associated with the post-hypercortisolemic state.

Sources of Research Support: NIH Grant K23 DK 082617 (to EBG), K24 DK 073040 (to PUF), CReFF award (to EBG), Mount Sinai Clinical Research Center award M01-RR-00071 and CTSA grant UL1RR029887.

Nothing to Disclose: EBG, WS, KDP, PUF

Pub # P3-345

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Adipogenesis & Adipocyte Function (1:30 PM-3:30 PM)

Title Modulation of Adipogenesis by EID1 Protein

Author String D Vargas, V Salgado, P Cortes, F Lizcano
La Sabana University, Chía, Colombia; Asociacion Medica de los Andes, Bogota, Colombia

Body

Introduction
The use of appropriate models for the study of adipogenesis and molecules that can modulate this process are of great importance due to increasing prevalence of obesity (1). Obesity is characterized by excess body fat, which leads to diseases such as hypertension, dyslipidemia, diabetes mellitus and cardiovascular risk (2). Effective therapeutic alternatives have become a strong research center today. We use pre-adipocytes cells 3T3-L1 and mesenchymal cells isolated from abdominal fat of women, to study the adipogenesis process of human pre-adipocytes cells and the phenotypic effect of EID1 when over-expressed in these two models. We found that EID1 decreases lipid accumulation in cells pre-adipocytes in both human and mouse models cells.

Objetive
Evaluate the phenotypic action of EID1 in differentiation of pre-adipocytes cells from human and mouse

Experimental procedures
3T3-L1 preadipocytes were grown in DMEM medium that contained 10% fetal bovine serum and human preadipocytes were obtained from adipose tissue samples from women with BMI less than 30 undergoing plastic surgery in the abdominal region. The samples were digested with 200U/mL of collagenase and centrifuged to 200g. The pellet were resuspenden in grow medium (3). To examine the over-expression effect of EID1, cells were grown to 90% of confluence and 1.6ug of EID1 in 4uL of lipofectamina were transfected. 48 hours after the transfection, was induced differentiation (3). Six days after treatment, cells were stained with oil red O and triglycerides levels quantified.

Results
The over-expression of EID1 decreased triglycerides in 3T3-L1 and human pre-adipocytes induced to differentiation. The phenotypic change were quantificate by removing the triglyceride oil red stained with isopropanol and then measure the absorbance at 510nm The results show significant difference \pm SE* $p < 0.001$ for mouse and $p < 0.05$ for human when EID1 is over-expressed.

Discussion
In this study, we describe to EID1 as a modulator of differentiation of pre-adipocytes. EID1 expression is decreased during adipogenesis of 3T3-L1 (4) cells indicating a role of this protein in regulating the early stages of differentiation. We hypothesize that EID1 activated proteins involving in caloric expenditure thereby reducing the accumulation of triglycerides by the adipocyte.

1. Li Mingfang, Cheung B. Pharmacotherapy for obesity. *Br J Clin Pharmacol* 2009;68:804.
2. Kahn S, Hull R, Utzschneider K. Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature* 2006;444:840.
3. Skurk T, Ecklebe S, Hauner H. Anovel technique to propagate primary human preadipocytes without loss of differentiation capacity. *Obesity* 2007;15:2925.
4. Lizcano F, Vargas D. EID1-induces brown-like adipocyte traits in white 3T3-L1 pre-adipocytes. *Biophy Biochem Res Commun*. 2010; 398:160.

Nothing to Disclose: DV, VS, PC, FL

Pub # P3-346

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Adipogenesis & Adipocyte Function (1:30 PM-3:30 PM)

Title The Regulatory Mechanism of Oxytocin on Adipogenesis in 3T3-L1 Adipocytes

Author String S Roh, S Song, Y Suzuki, K So, Y Kikuchi, A Astrid, C Chen, K Katoh
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Body Oxytocin, synthesized in hypothalamic neurons and transported down in axons to the posterior pituitary where it is secreted into blood circulation, plays an important role as an inducer of uterine contraction during parturition and of milk ejection after giving birth. Oxytocin receptor (OXTR) is expressed by the myoepithelial cells of the mammary gland, and in both the myometrium and endometrium of the uterus at the end of pregnancy. OXTR expression has also been observed in peripheral tissues including adipose tissues. It was reported that oxytocin inhibits the adipogenic differentiation in human mesenchymal stem cells [1]. However, the molecular mechanisms of oxytocin action in regulating adipogenic program remain largely unknown. The aim of this study was to confirm expression of OXTR in adipose tissues and to investigate the regulatory mechanism of oxytocin action on adipogenic and lipolytic processes in 3T3-L1 adipocytes. OXTR gene expression was induced during induction of adipogenic differentiation and significantly increased along adipocyte differentiation. OXTR mRNA expression was markedly elevated in adipose tissues of mice fed by a high-fat diet compared to mice fed with control diet. To investigate whether oxytocin modified the expression of adipogenic and lipolytic genes, 3T3-L1 adipocytes were treated with oxytocin (10^{-7} M) for 24 hr. Such treatment significantly up-regulated OXTR, Glut-1 and Glut-4 mRNA expression. In addition, TNF- α , IL-6, and chemerin mRNA levels were significantly increased in the 3T3-L1 adipocytes by the oxytocin treatment. Meanwhile, leptin, PPAR- γ 2, and adipogenin mRNA levels were decreased by the oxytocin treatment. These data suggests that oxytocin is one of the regulatory factors of adipogenesis, up-regulating the lipolytic genes expression and down-regulating the adipogenic genes expression through OXTR in adipocytes.

[1] Christian E, Armelle B, Helene B, Veronique B, Nicole W, Marcel S, Laure A Z, Florence M, Emmanuel L, Zlatko T, Georges C, Liana E, Gerard A, Claud B, Christian D, Ez-zoubir A. (2008). "Oxytocin controls differentiation of human mesenchymal stem cells and reverses osteoporosis". STEM CELLS 26:2399-2407.

Nothing to Disclose: SR, SS, YS, KS, YK, AA, CC, KK

Pub # P3-347

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Adipogenesis & Adipocyte Function (1:30 PM-3:30 PM)

Title Circadian Rhythm Gene *Per3* Is an Inhibitor of the Adipocyte Cell Fate

Author String BJ Feldman, MJ Costa, AY So, K Kaasik, KC Krueger, ML Pillsbury, Y-H Fu, LJ Ptacek, KR Yamamoto
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Body Glucocorticoids rapidly and robustly induce cell fate decisions in various multipotent cells, although the precise mechanisms of these important cellular events are not understood. Here we showed that glucocorticoids repressed *Per3* expression, and that this repression was critical for advancing mesenchymal stem cells to the adipocyte fate. Exogenous expression of *Per3* inhibited adipogenesis, whereas knocking out *Per3* enhanced that fate. Moreover, we found that *PER3* formed a complex with *PPAR γ* , and inhibited *PPAR γ* mediated transcriptional activation via *Ppar γ* response elements. Consistent with these findings, *Per3* knockout mice displayed alterations in body composition, with both increased adipose and decreased muscle tissue compared to wild-type mice. Our findings identify *Per3* as potent mediator of cell fate that functions by altering the transcriptional activity of *PPAR γ* .

Sources of Research Support: Funded by The NIH Director's New Innovator Award Program 1-DP2-OD006740 (to B.J.F.)

Nothing to Disclose: BJF, MJC, AYS, KK, KCK, MLP, Y-HF, LJP, KRY

Pub # P3-348
Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Adipogenesis & Adipocyte Function (1:30 PM-3:30 PM)
Title Effects of Ubiquitous and Liver-Specific Expression of Human CIDE-A Expression in Transgenic Mice
Author String B Kelder, DE Berryman, N Linn, M Tamowski, EO List, JJ Kopchick
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Body CIDE-A (cell-death-inducing DFF45-like effector A) is a member of a family of proteins that includes CIDE-B and CIDE-C (FSP27). These proteins have been shown to associate with the surface of lipid droplets within cells and are believed to function in the regulation of lipid metabolism. In support of this theory, CIDE-A gene disrupted mice have been shown to exhibit a lean phenotype and are resistant to diet-induced obesity (1). We have previously demonstrated that CIDE-A expression levels are reduced in subcutaneous white adipose tissue of obese mice (2). These obese mice also demonstrate a concomitant increase in CIDE-A expression in liver that is correlated with liver steatosis (3). We have generated transgenic mice that express the human CIDE-A cDNA under the control of the mouse metallothionein-I transcriptional regulatory element (TRE). This TRE directs ubiquitous and constitutive transgene expression. We have characterized these mice with respect to body weight, fasting blood glucose and plasma insulin levels, glucose tolerance and by determining body composition (fat, body fluid and lean tissue) by NMR. At 1 year of age, female CIDE-A transgenic mice have a significantly lower body weight, lower fasting blood glucose and plasma insulin levels and increased glucose tolerance compared to their nontransgenic littermates. Body composition analysis indicates that these mice also have significantly less fat compared to control mice. We have found that the difference in fat mass between transgenic and control mice was not a result of decreased fat in any one particular depot. Rather, the CIDE-A mice have a decreased amount of fat in all adipose depots tested (subcutaneous, perigonadal, retroperitoneal, mesenteric and brown adipose). To further delineate CIDE-A's role in the regulation of lipid metabolism, we have generated a tissue-specific expression plasmid in which transgenic human CIDE-A cDNA expression is under the control of the mouse albumin TRE. Transgenic mice containing this fusion plasmid will express CIDE-A only in the liver.

1. Zhou Z, Yon Toh S, Chen Z, Guo K, Ng CP, Ponniah S, Lin SC, Hong W, Li P. Cidea deficient mice have lean phenotype and are resistant to obesity. *Nat. Genet.* 35:49-56, 2003.
2. Kelder B, Boyce K, Kriete A, Clark R, Berryman DE, Nagatomi S, List EO, Braughler M, Kopchick JJ. CIDE-A is expressed in liver of old mice and in type 2 diabetic mouse liver exhibiting steatosis. *Comp. Hepatol.* May 1;6:4, 2007.
3. Kelder B, Berryman DE, Clark R, Li A, List EO, Kopchick JJ. CIDE-A gene expression is decreased in white adipose tissue of growth hormone receptor/binding protein gene disrupted mice and with high-fat feeding of normal mice. *Growth Horm IGF Res.* 17(4):346-351, 2007.

Sources of Research Support: NIH (Grant 1R15DK083729-01A1) and by the State of Ohio's Eminent Scholar Program that included a gift from Milton and Lawrence Goll.

Nothing to Disclose: BK, DEB, NL, MT, EOL, JJK

Pub # P3-349

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Adipogenesis & Adipocyte Function (1:30 PM-3:30 PM)

Title DRG2 Regulates Lipid Homeostasis through Modulating Peroxisome Proliferator-Activated Receptor-gamma Activity

Author String JG Kim, BS Park, BJ Lee
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Body Developmentally regulated GTP-binding protein 2 (DRG2) is an uncharacterized member of the Obg family, an evolutionary branch of GTPase superfamily proteins. There are at least two distinct members, DRG1 and DRG2. They both are widely expressed in human and mouse tissues and share very similar pattern of expression. Although pattern of DRG2 expression in mammals has been reported, its physiological function is unclear. We generated DRG2 transgenic (TG) mice overexpressing DRG2 to characterize its physiological roles in vivo. DRG2 TG mice showed interesting phenotype associated with lipid metabolism: increased body fat content with significantly changed expression of genes involved in lipogenesis and lipolysis; increased serum concentration of metabolic parameters such as insulin and leptin with overt glucose intolerance. Moreover, we demonstrated that DRG2 is a critical modulator for PPAR-gamma activation and function in lipid homeostasis. These findings suggest that DRG2 is an important regulator for adipocyte differentiation and lipid metabolism.

Nothing to Disclose: JGK, BSP, BJL

Pub # P3-350

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Adipogenesis & Adipocyte Function (1:30 PM-3:30 PM)

Title HIV-1 Accessory Protein Viral Protein R (Vpr) Inhibits PPAR γ Signaling To Disrupt Lipid Metabolic Pathways *In Vivo*: Implications for the Pathogenesis of HIV Lipodystrophy

Author String N Agarwal, SG Patel, O Toni, D Iyer, J Kopp, RV Sekhar, T Kino, DE Lewis, A Balasubramanyam
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Body The pathogenesis of adipocyte defects and lipid kinetic abnormalities in HIV-associated lipodystrophy is unclear. The HIV-1 accessory protein Viral protein R (Vpr) acts in vitro as a corepressor of PPAR γ , a critical regulator of lipid metabolism and adipose insulin sensitivity. We investigated whether Vpr can cause adipocyte and lipid metabolic dysfunction in vivo by interfering with PPAR γ -mediated gene transcription, using transgenic mice expressing Vpr under control of the PEPCK promoter in liver, adipose tissues and kidney. Vpr-Tg mice or WT littermates were given the PPAR γ agonist rosiglitazone or vehicle IP for 14 d from age 12 weeks. There were 16 mice (8 M, 8 F) in each of 4 groups (WT+vehicle, WT+rosi, Vpr-Tg+vehicle, Vpr-Tg+rosi). Animals were sacrificed after a 12 h fast, and expression of PPAR γ target genes critical for fat metabolism was quantified in liver and perigonadal fat (PGF) by qPCR, and plasma adiponectin by ELISA. Group results were compared using two-way paired t-tests. PPAR γ mRNA levels were increased in PGF of WT mice by rosi treatment (+28% compared to vehicle, P=0.01). In contrast, PPAR γ mRNA levels were downregulated in PGF of Vpr-Tg compared to WT (-36%, P=0.01) and this effect was not reversed by rosi treatment. Similarly, adiponectin mRNA was modestly upregulated by rosi in PGF of WT (+17%) but downregulated in PGF of Vpr-Tg mice (-32%, P=0.01) and not rescued by rosi. Concomitantly, levels of plasma adiponectin protein were increased by rosi in WT (+70%, P=0.0001) but decreased in Vpr-Tg mice (-30%, P=0.01). mRNA for the fatty acid binding protein aP2 was also downregulated in PGF of Vpr-Tg (-35%, P=0.001) and not rescued by rosi. In liver of Vpr-Tg, multiple genes related to fatty acid transport and oxidation were downregulated: CPT1 (-23%, P=0.03), AOX (-23%, P=0.01), LCAD (-27%, P=0.04), MTP (-34%, P=0.04), and UCP-2 (-24%, P=0.02); in all cases, rosi did not reverse the Vpr effect. There were no gender differences in these mRNA results; however, male Vpr-Tg showed 20-30% diminution in adipose mass whereas female Vpr-Tg showed 50-70% increase (all depots). HIV-1 Vpr inhibits PPAR γ signaling in fat and liver to disrupt fatty acid transport and oxidation, and blunt expression of the insulin-sensitizing adipokine adiponectin. In conjunction with emerging data from metabolic studies in Vpr-Tg mice, these data indicate a novel and significant role for Vpr in the pathogenesis of HIV lipodystrophy.

Sources of Research Support: NIH Grant RO1 DK081553 to AB.

Nothing to Disclose: NA, SGP, OT, DI, JK, RVS, TK, DEL, AB

Pub # P3-351

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Adipogenesis & Adipocyte Function (1:30 PM-3:30 PM)

Title Core Adipocyte Genes Are Regulated by Multiple Conserved and Species-Specific PPAR γ Binding Sites

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Body The nuclear receptor transcription factor PPAR γ is the master regulator of adipogenesis, regulating gene networks essential to adipocyte biology. Chromatin immunoprecipitation (ChIP) of PPAR γ has previously identified thousands of binding sites in the genome of mouse 3T3-L1 adipocytes. Remarkably, in similar analysis of PPAR γ binding sites in human SGBS adipocytes (kindly provided by M. Wabitsch), we find that most of the murine PPAR γ binding sites are not highly conserved across species. Only ~10% of binding events occur at syntenic locations in both genomes, although the same canonical binding motifs are utilized. The presence of interspecies sequence conservation or even binding motifs predict experimental binding poorly, showing limitations of *in silico* analysis. Nevertheless, gene-based analysis of the two species reveals sharing of ~50% of genes with nearby PPAR γ binding, and these shared genes function in fat-specific metabolic pathways that are conserved across species. Combining cistrome and gene expression analysis, we identify here a core set of genes that are induced in adipogenesis in both species and surrounded by multiple PPAR γ binding sites, some of which are conserved while most are species-specific. Genes that are only up-regulated in one species often have nearby binding sites in only that species, indicating that species-specific binding sites are functional in regulating nearby genes. These species-specific genes do not show strong enrichment for adipocyte pathways, yet they often reside in close genomic proximity to core adipocyte genes. This suggests that species-specific genes may be mere bystanders that are coincidentally co-regulated in a species-specific manner by PPAR γ sites that evolved to regulate the core genes. Thus, core adipogenic genes are coordinately regulated in multiple species and have clusters of surrounding PPAR γ sites, some of which are species-specific and may regulate nearby species-specific bystander genes that are likely not essential for adipocyte biology.

Sources of Research Support: Nuclear Receptor Signaling Atlas U19DK/HL/ES 62434 (MAL) Training grant T32 DK007314 (RES).

Nothing to Disclose: RES, LJE, GT, KHK, MAL

Pub # P3-352
Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Adipogenesis & Adipocyte Function (1:30 PM-3:30 PM)
Title Steroid Formation in Adipocytes: Biosynthesis and Function
Author String J Li, V Papadopoulos
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Body As one of the most important endocrine organs in the human body, adipose tissue plays an important role in steroid metabolism, growth and reproductive function. There is evidence that the transcription of genes encoding the Translocator Protein TSPO (18-kDa) and its endogenous ligand Diazepam Binding Inhibitor (DBI) is induced during 3T3-L1 mouse preadipocyte differentiation. TSPO is a cholesterol-binding protein mediating the initial rate-limiting step of steroidogenesis, the transport of the substrate cholesterol from intracellular stores into mitochondria where it is metabolized to pregnenolone by CYP11A1. Binding of DBI to TSPO accelerates cholesterol transport into mitochondria and steroid formation. These data suggest that in addition to their known ability to metabolize exogenous steroids, adipocytes may be able to synthesize steroids *de novo*. Gene and protein expression studies demonstrated that the expression level of major components of the steroidogenic machinery, such as TSPO, the steroidogenic acute regulatory protein STAR, and the steroidogenic enzymes CYP11A1 and CYP17A1 mediating the formation of pregnenolone and androgens increased during 3T3-L1 differentiation induced by exposure of the preadipocytes to dexamethazone, insulin and 3-isobutyl-1-methyl-xanthine. Moreover, 3 β -hydroxysteroid dehydrogenase, the enzyme critical for the formation of progesterone from pregnenolone, was also found in preadipocytes and adipocytes but its expression was not affected during adipogenesis. Treatment of the cells with the TSPO drug ligand PK 11195 increased 3T3-L1 adipocyte differentiation. In contrast, treatment of the cells with the CYP11A1 inhibitor aminoglutethimide resulted in reduction of lipid droplet formation and inhibition of 3T3-L1 differentiation, suggesting a functional role of the steroid biosynthesis pathway in adipocyte differentiation. Metabolic studies were undertaken to examine the ability of 3T3-L1 adipocytes to synthesize steroids *de novo* from radiolabeled mevalonate, the direct precursor of cholesterol. Separation by HPLC of organic extracts from 3T3-L1 adipocytes, incubated with radiolabeled mevalonate, indicated the formation of radiolabeled steroid products. The identity of the steroids formed is under investigation. Taken together these data suggest that 3T3-L1 adipocytes have the potential to synthesize steroids *de novo* and the endogenous steroids formed play a role in adipocyte differentiation.

Sources of Research Support: CIHR.

Nothing to Disclose: JL, VP

Pub # P3-353

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Adipogenesis & Adipocyte Function (1:30 PM-3:30 PM)

Title New Key Players in Adipogenesis from GWAS? Relevance of Genes Found Associated with Obesity in Genome-Wide Association Studies in Human Adipocytes

Author String F Bernhard, K Landgraf, P Buttner, D Friebe, W Kiess, A Korner
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Body The expansion of adipose tissue is based on a complex system of transcriptional factors, adipocytokines and other factors. Recent Genome Wide Association studies (GWAs) identified new genes associated with obesity. Most of these were set in context with obesity for the first time and their relevance for the development of adipose tissue is largely unknown. The aim of our study was to characterize the respective genes in adipocytes by expression- and functional analyses in vitro.
We selected eight genes (*BDNF*, *MAF*, *MTCH2*, *NEGR1*, *NPC1*, *PTER*, *SH2B1*, *TMEM18*) from six GWAs based on scoring for significance ($p \leq 10^{-7}$), odds-ratio, intragenic position of the variants, independent replication and whole genome expression data. We compiled human tissue expression arrays to assess the expression pattern in metabolic, endocrine, neural and other tissues. All of the candidate genes were basally expressed in adipose tissue. Expression analyses of the genes during adipocyte differentiation using the human adipocyte model SGBS revealed a modest up regulation of *MAF*, *MTCH2*, and *NEGR1* and a threefold down regulation of *BDNF*. There was no obvious regulation of *NPC1*, *PTER*, *SH2B1* and *TMEM18* during adipocyte differentiation. To investigate the regulation of the genes by metabolic factors, we analyzed the effect of insulin, dexamethasone, IGF-1 and the β -agonist isoproterenol on the gene expression in both, adipocytes and preadipocytes. *NEGR1* was up regulated by dexamethasone (3.217 ± 0.62 fold, $p=0.0117$) and down regulated by insulin (1.723 ± 0.11 fold, $p=0.0085$) and IGF-1 (1.586 ± 0.01 fold, $p<0.0001$) in adipocytes. Likewise, *BDNF* is significantly down regulated after stimulation with insulin (1.678 ± 0.02 fold, $p<0.0001$), dexamethasone (1.500 ± 0.06 fold, $p=0.0012$) and IGF-1 (2.019 ± 0.04 fold, $p<0.0001$) in adipocytes. *MAF* gene expression was inhibited by dexamethasone in preadipocytes (2.849 ± 0.15 fold, $p=0.0048$) and adipocytes (3.326 ± 0.12 fold, $p=0.0010$). Finally, to address the question whether the candidate genes play a role in adipogenesis we used RNA-interference to knock down gene function. Knock down of *BDNF*, *MTCH2*, *NEGR1* and *TMEM18* gene function resulted in an up to 30% reduced triglyceride accumulation in mature adipocytes whereas the other candidate genes had no effect on adipogenesis.
The regulation and their importance for adipocyte differentiation as well as the response to adipogenic factors indicates a role of *BDNF*, *MTCH2*, *NEGR1* and *TMEM18* as new players in adipogenesis.

Nothing to Disclose: FB, KL, PB, DF, WK, AK

Pub # P3-354

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Adipogenesis & Adipocyte Function (1:30 PM-3:30 PM)

Title Modeling Dunnigan-Type Familial Partial Lipodystrophy Using Patient-Specific Induced Pluripotent Stem Cells

Author String Y-K Lee, T Ahfeldt, FH Lau, F Xia, CA Cowan
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Body Dunnigan-type familial partial lipodystrophy syndrome type 2 (FPLD2) is an autosomal dominant genetic disorder caused by a number of mutations in the LMNA gene. The major clinical feature of FPLD2 is fat loss in the limbs and trunk, with elevated fat storage in the neck and face. Due to the selective fat loss, patients exhibit metabolic dysfunction such as insulin resistance, glucose intolerance, and accumulation of plasma triglycerides. They often develop diabetes mellitus, hypertriglyceridemia, and early-onset atherosclerosis, which are also found in individuals with morbid obesity. While the physiological consequences of LMNA mutations have been described, very little about the molecular events underlying the disease is known because of the absence of an accurate model system. Human adipose is easily obtained, however primary adipocytes are difficult to maintain in culture and are not amenable to expansion. As a consequence, *in vitro* systems for understanding mature primary adipocyte function do not exist. In an attempt to better understand the pathophysiology of FPLD2 and the role of LMNA in adipose tissue, we created the human cell-based model of FPLD2. First, we generated induced pluripotent cells (iPSC) from patients with FPLD2 by reprogramming their fibroblasts with four transcription factors (OCT4, SOX2, KLF4, and cMyc). The patient-specific human iPSCs exhibit the pluripotent state similar to human embryonic stem cells (ESC), demonstrated by endogenous gene expression (qRT-PCR and immunostaining) and differentiation potential (embryoid body and teratoma formation for three lineage differentiation). Second, we developed a novel protocol for differentiating the iPSCs into adipocytes, which by inducing the expression of PPARG2 in iPSC-derived CD73+ cells, to elucidate the molecular events that lead to the development of FPLD2. We found decreased efficiency of differentiation and maturity in FPLD2 iPSC-derived adipocytes compared to control. Gene expression also showed that adipogenic genes such as GLUT4 and Adiponectin were down-regulated in FPLD2 iPSC-derived adipocytes. Further, we are exploring the cellular processes by lipolysis, fatty acid oxidation, insulin resistance, and glucose transport assays. Our human cell-based model of FPLD2 will provide a valuable system for a limitless source for human-derived adipocytes to study disease progression and drug development.

Nothing to Disclose: Y-KL, TA, FHL, FX, CAC

Pub # P3-355

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Adipogenesis & Adipocyte Function (1:30 PM-3:30 PM)

Title WISP1 Mediates Bmp3-Stimulated Mesenchymal Stem Cell Proliferation

Author String H Guan, K Yang
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Body Obesity is a consequence of increased adipose tissue mass resulting from adipocyte hyperplasia and/or hypertrophy. Adipocytes are derived from mesenchymal stem cells (MSCs) through adipogenesis; a process that involves three key steps, including proliferation, commitment, and terminal differentiation. The factors that promote adipogenesis remain largely unknown. Previously we demonstrated that bone morphogenetic protein 3 (BMP3), a member of the transforming growth factor β (TGF- β) superfamily, potently stimulates MSC proliferation. In the present study, we investigated the molecular targets of BMP3. To uncover downstream target genes in the BMP3 signaling pathway, we conducted DNA microarray analysis on MSCs treated with and without BMP3, and identified WISP1 (WNT1-inducible signaling pathway protein-1) as a differentially expressed gene, whose expression was up regulated 3.7-fold by BMP3. WISP1 (also known as CCN4) is a member of the CCN (CYR61/CTGF/Nov) family of growth factors, and has been shown to promote proliferation of cardiac fibroblasts, lung epithelial cells, and osteogenic cells. WISP1 is also an oncogene, and a pro-survival factor. Therefore, we tested the hypothesis that WISP1 mediates BMP3 stimulation of MSC proliferation. Using qRT-PCR and western blot analysis, we showed that BMP3 increases the expression of WISP1 mRNA and protein as early as 6 h following BMP3 treatment in MSCs. Importantly, the up-regulated WISP1 expression preceded BMP3-induced MSC proliferation, as determined by [³H]-thymidine incorporation. Furthermore, treatment of MSCs with recombinant BMP3 led to a concentration-dependent increase in [³H]-thymidine incorporation with a maximal effect at 1 [μ]g/ml (~3-fold increase). In addition, siRNA-mediated knockdown of WISP1 expression attenuated BMP3-induced MSC proliferation. Taken together, our present findings reveal WISP1 as a novel target of BMP3, and suggest that a BMP3/WISP1 signaling pathway may play a key role in MSC proliferation, thus providing new insights into not only BMP3 function but also adipogenesis.

Sources of Research Support: Canadian Institutes of Health Research.

Nothing to Disclose: HG, KY

Pub # P3-356

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Adipogenesis & Adipocyte Function (1:30 PM-3:30 PM)

Title Nitric Oxide (NO) Induces Both Glucose Uptake and Mitochondrial Biogenesis in Adipose Tissue

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Body Among the different mechanisms implicated in the pathogenesis of insulin resistance, the existence of a reduction in mitochondrial content and function along with an impairment in eNOS enzymatic activity has been recently highlighted. Furthermore, it has been reported that mitochondria are defective not only in muscles, but also in adipose tissue of insulin resistant obese subjects and in patients with type 2 diabetes and this deficit may contribute to the impairment of signal transduction and substrate transport and oxidation. Preliminary results obtained in our lab suggested that in eNOS $-/-$ mice a decrease in mtDNA and mRNA of mitochondrial specific genes along with a decreased tissue insulin glucose uptake are present in adipose tissue after exercise training. We aimed therefore to study the effects of a nitric oxide donor (DETA-NONOate) on the mitochondrial biogenesis and glucose uptake in 3T3-L1 preadipocytes.

Material and Methods: In our study fully differentiated 3T3-L1 cell line were utilized. The gene expression analysis of 3T3-L1, treated with either NO donor, DETA-NONOate (100[μ M] for 72 hours) or vehicle alone was performed by Real Time PCR method. The analyzed genes which are involved in the regulation of mitochondrial biogenesis (transcriptional factor: PGC1 α , TFAM, NRF1) and genes coding for protein of the mitochondrial respiratory chain (COXIV) and activity (MFN2). We measured also the capability of basal and insulin stimulated glucose uptake of 3T3-L1 cells after a DETA-NONOate treatment using the [3 H]-2-Deoxyglucose uptake method. We evaluated mitochondrial morphology with 100nM of Mitotracker Green FM dye, a reliable indicator of mitochondrial levels in live cells.

Results and Conclusions: The present results confirm that the exercise mediated increase in adipose tissue mitochondrial biogenesis and insulin sensitivity could be linked together. In fact, we observed an increased expression of genes involved in mitochondrial biogenesis in DETA-NONOate treated cells in comparison with vehicle treated cells. Interestingly the treatment with DETA-NONOate induced an increase in both basal and insulin stimulated glucose uptake. The MTG staining showed an increased of mitochondrial area and elongation of DETA-NONOate treated cells. These results suggest that nitric oxide is a key molecule in the regulation of energy balance and glucose metabolism, through activation of mitochondrial biogenesis.

Nothing to Disclose: RV, MG, ET, AC, EN

Pub # P3-357

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Adipogenesis & Adipocyte Function (1:30 PM-3:30 PM)

Title Phosphodiesterase Type 5 (PDE5) in Human Adipose Tissue: Site-Specific Expression and Role in Adipocyte Function

Author String A Armani, V Marzolla, A Antelmi, A Feraco, M Calanchini, C Mammi, GMC Rosano, M Caprio, A Fabbri IRCCS San Raffaele Pisana, Rome, Italy; CTO- A Alesini Hospital, University Tor Vergata, Rome, Italy

Body Phosphodiesterase type 5 (PDE5) selectively hydrolyzes cyclic guanosine monophosphate (cGMP) and has been detected in many tissues including adipose tissue. In adipose tissue, cGMP regulates the process of lipolysis and PDE5 activity might play an important role in modulating the lipolytic process. We investigated site-specificity of PDE5 expression in anatomically distinct human adipose depots and studied the possible role of PDE5 in human adipocyte function. Adipocytes and preadipocytes were purified from subcutaneous and epicardial visceral fat of patients undergoing surgery for valvular heart disease. We also purified adipose cells from mesenteric adipose tissue of patients undergoing abdominal surgery for inguinal herniation. We only selected patients aged between 40 and 60 years, with normal body weight and ejection fraction, without oncologic or coronary artery disease. PDE5 expression has been evaluated by Real-time PCR. In cultures of visceral preadipocytes, PDE5 mRNA levels decreased during adipose conversion. After 10 days of differentiation, adipose cells expressed PDE5 mRNA levels comparable to those measured in mature adipocytes. Interestingly, we observed that epicardial adipocytes differentiated in vitro showed higher levels of PDE5 than subcutaneous adipocytes from the same patient. Atrial natriuretic peptide (ANP)-induced lipolysis in terminally differentiated adipocytes was not modulated by the PDE5 specific inhibitor sildenafil, excluding a role for PDE5 in regulating ANP-mediated lipolysis. More interestingly, acute exposure of mature adipocytes to PDE5 inhibitors treatment increased mRNA and protein expression of aromatase with a parallel increase of estradiol concentrations in culture medium, indicating a role for PDE5 in modulating estradiol production and release from adipose tissue. In order to analyse a potential role of PDE5 during adipose differentiation, we also treated differentiating visceral preadipocytes with sildenafil, observing an increase in triglyceride accumulation, with a parallel up-regulation of mRNA leptin expression; more important, the pro-inflammatory cytokine TNF α was down-regulated by sildenafil. Given the importance of adipose tissue as pivotal player in controlling the function of different organs in the context of energy homeostasis, a better elucidation of the role of PDE5 and its pharmacological inhibition could help to clarify the pathophysiology of obesity and metabolic syndrome.

Nothing to Disclose: AA, VM, AA, AF, MC, CM, GMCR, MC, AF

Pub # P3-358

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diet, Nutrients & Obesity: Impact on Inflammation & Adipose Tissue (1:30 PM-3:30 PM)

Title Hypothalamic SIRT1 Regulates Prohormone Convertase 2 in a Nutrient-Dependent Manner

Author String NE Cyr, R Beckman, RC Stuart, EA Nillni
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Body Sirt1 is an evolutionarily conserved NAD \pm -dependent deacetylase that has generated excitement because it regulates important physiological functions such as cellular differentiation, apoptosis, metabolism, and aging. More recently, Sirt1 has received considerable attention as a critical nutrient/energy sensor in central and peripheral tissues. We recently showed that pharmacological inhibition or siRNA silencing of Sirt1 present in the arcuate nucleus (ARC) of the hypothalamus decreases both food intake and body weight gain (PLoS One. 2009 Dec 15;4(12):e8322.PMID: 20020036). Sirt1 within the hypothalamic ARC was shown to regulate proopiomelanocortin and the central melanocortin system. In the present study, we investigated whether Sirt1 also plays a role in another hypothalamic region that controls energy balance, the paraventricular nucleus (PVN). Here, we show in rats that Sirt1 protein levels were higher in diet-induced obese (DIO) rats kept on high fat diet for 12 weeks compared to lean counterparts. Elevated Sirt1 in the PVN increased the levels of prohormone convertase 2 (PC2), which is the enzyme responsible for the conversion of pro-corticotropin-releasing hormone (pro-CRH) to the bioactive form of CRH peptide. This effect of Sirt1 on PC2 was exacerbated in the PVN of DIO rats, which further support a role for Sirt1 regulation of energy balance and body weight. CRH has well known effects on food intake and increased levels of bioactive CRH in the PVN over time activate the hypothalamic-pituitary-adrenal (HPA) axis such that baseline (i.e. not stress-induced) circulating glucocorticoids (GCs) is chronically elevated, which alters metabolism and increases food-intake in a diet-dependant manner. Overall, results indicate that Sirt1 in the PVN regulates a key enzyme that processes pro-CRH, which in turn regulates metabolism.

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Nothing to Disclose: NEC, RB, RCS, EAN

Pub # P3-359

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diet, Nutrients & Obesity: Impact on Inflammation & Adipose Tissue (1:30 PM-3:30 PM)

Title *Ad Libitum* Consumption of a High-Fat, Cafeteria-Style Diet Decreases the Effectiveness of Body-Weight Reductions Induced by Vertical Sleeve Gastrectomy in Rats

Author String M Bielohuby, K Stemmer, J Berger, K Smith, K Parks, J Ramisch, PT Pfluger, MH Tschop, RJ Seeley, M Bidlingmaier
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Body Vertical sleeve gastrectomy (VSG) has been shown to reduce bodyweight (BW) in rats with diet induced obesity (DIO), but also in obese humans. Only little attention has been paid how the consumption of different diets affects BW and body composition after VSG. We therefore investigated the effects of feeding 3 differentially composed diets to rats after either VSG or sham surgery. **Methods:** VSG was performed in male Long-Evans rats with DIO and obese controls were sham operated (n[ge]6/group). After a 3-day recovery phase on a liquid diet, rats were fed ad libitum either a standard rodent chow diet (CH; energetic density in metabolizable energy (ME) and diet composition as a percentage of ME (fat/protein/CHO): 17.6MJ/kg; 16.7/19/64.3), or a cafeteria-style, high-fat diet (Caf; 22.2MJ/kg; 61.9/18.7/19.4) or a low-carbohydrate, high fat diet (LCHF; 27.7MJ/kg, 78.7/19.1/2.2). BW and food intake were measured every second day. After 4 weeks on the respective diets, body composition was analyzed by an NMR instrument. **Results:** After 4 weeks, BW-gain in all rats undergoing VSG was more than 20% reduced when compared to sham controls (p<0.05). Irrespective of the diet, cumulative 4-week energy intake was significantly lower after VSG when compared to respective sham controls (p<0.05). Sham operated rats fed LCHF consumed most energy, followed by rats fed Caf. In contrast, after VSG cumulative energy intake was highest in rats fed Caf, but not significantly different between CH and LCHF (CH sham: 9.1±0.3MJ; LCHF sham: 11±0.6MJ; Caf sham: 10.2±0.8MJ; CH-VSG: 5.5±0.3MJ; LCHF-VSG: 5.3±0.2MJ; Caf-VSG: 6.7±0.4MJ; CH-VSG vs. Caf-VSG: p<0.05). BW gain was not significantly different between CH-VSG and LCHF-VSG groups, but significantly higher in the Caf-VSG group (p<0.05). NMR analysis revealed that lean body mass was not different between VSG groups. However, fat mass was higher (p<0.01) in Caf-VSG compared to CH-VSG (CH-VSG: 36.2±5.8g; Caf-VSG: 68.5±6.5g; LCHF-VSG: 46.3±3.6g).
In conclusion, ad libitum consumption of a high fat cafeteria diet reduced the effectiveness of VSG in rats. Surprisingly, consumption of the LCHF diet -which was even more energy dense than the cafeteria diet - did not negatively affect the BW outcome of VSG. Our results suggest that 1) VSG should be accompanied by respective lifestyle modifications including the avoidance of high fat, cafeteria-style diets and 2) VSG may reduce fat palatability, thereby leading to reduced consumption of LCHF diets.

Nothing to Disclose: MB, KS, JB, KS, KP, JR, PTP, MHT, RJS, MB

Pub # P3-360

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diet, Nutrients & Obesity: Impact on Inflammation & Adipose Tissue (1:30 PM-3:30 PM)

Title Regulation of Adipocyte Differentiation and Metabolism by Fructose and GluT5

Author String L Du, AP Heaney
David Geffen School of Medicine, University of California, Los Angeles, CA

Body Adipose tissue is an important endocrine organ that is crucial for whole-body insulin sensitivity and energy homeostasis. The differentiation of preadipocytes to mature adipocytes is a highly regulated process that is associated with a well characterized sequential alteration in expression of several genes including PPAR γ , C/EBP α and GluT4. Previous studies have reported that high refined fructose intake may promote visceral adiposity in overweight or obese humans, but the potential mechanism remains unclear. We demonstrated that addition of fructose (55-5500 μ M) to differentiation medium of 3T3-L1 cells resulted in a dose-dependant enhancement of adipogenesis and induction of the adipocyte-related genes PPAR γ , C/EBP α and GluT4. Our results also demonstrated that GluT5, the key fructose transporter, was expressed during the differentiation of 3T3-L1 cells but was not expressed in mature adipocytes. Treatment of 3T3-L1 cells with the GluT5 inhibitor (1-O-Benzyl-2-N,3-O-carbonyl- α -L-sorbofuranosylamine, L-Bn-OZO) decreased adipocyte differentiation. Overexpression of GluT5 repressed differentiation and knock-down of GluT5 induced differentiation in 3T3-L1 preadipocytes and altered PPAR γ and C/EBP α expression. Taken together, these results suggest that fructose and GluT5 play an important role in regulating adipose differentiation and metabolism.

Nothing to Disclose: LD, APH

Pub # P3-361

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diet, Nutrients & Obesity: Impact on Inflammation & Adipose Tissue (1:30 PM-3:30 PM)

Title Obesogenic Diet-Induced Changes of 11 β -Hydroxysteroid Dehydrogenase 1 Gene Expression in Subcutaneous Depot and Liver, in Female Sprague-Dawley Rats

Author String K Simonyte, I Soderstrom, A Sjodin, T Olsson
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Body Obesity and its complications have clear links to availability of glucocorticoids. 11 β hydroxysteroid dehydrogenase 1 (11 β HSD1) interconverts corticosterone (cortisol in humans) and 11-dehydrocorticosterone (cortisone in humans) regulating its access to glucocorticoid receptors. Overexpression of 11 β HSD1 in adipose creates a complete metabolic syndrome, while hepatic 11 β HSD1 overexpression leads to fatty liver, hypertension and insulin resistance without fat accumulation. 11 β HSD1 knockout mice have reduced visceral fat accumulation and are protected from hyperglycemia and dyslipidemia when exposed to a high-fat diet. However, these observations are based on monogenic-obese rodents and do not reflect the polygenic nature of obesity. This study aimed to investigate the impact of high-energy diet (HED) on peripheral 11 β HSD1 in absence of specific genetic background, using female Sprague-Dawley rats. Ninety Sprague-Dawley female rats were randomly divided into two groups: 50 rats assigned for HED and 40 rats - for a control diet (CD). For 20 weeks, every second week 5 rats from HED group and 4 from CD group were sacrificed; blood, subcutaneous adipose and liver samples were collected. Circulating levels of estradiol, corticosterone, insulin, leptin and adiponectin were measured. 11 β HSD1, H6PDH, PEPCK and A-ring reductases mRNA levels were analyzed. Weight did not differ between the groups, however HED fed rats had significantly higher serum leptin, but lower adiponectin concentrations compared to CD rats (diet effect: $p < 0.05$ for leptin and $p < 0.001$ for adiponectin, respectively). Subcutaneous 11 β HSD1, H6PDH and PEPCK mRNA levels were significantly higher in HED rats vs. CD rats already from week two. From week 14, the expression pattern of these genes significantly changed to the opposite (interaction effect $p < 0.01$, for all genes). In the liver, HED induced a down-regulation of 11 β HSD1 and A-ring reductases mRNA levels (diet effect $p < 0.001$, for all genes). An obesogenic diet, even without an increase in weight, leads to an unfavorable metabolic profile and changes in 11 β HSD1 expression in adipose tissue and liver. Interestingly, early increase is followed by a down-regulation of SAT 11 β HSD1. Concomitantly, chronic HED feeding had a downregulatory effect on hepatic 11 β HSD1 and A-ring reductases. These data suggest a strong influence of high-energy diet on 11 β HSD1 in SAT and liver. Notably, these effects differed between short-term and chronic diet modulation.

Nothing to Disclose: KS, IS, AS, TO

Pub # P3-362

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diet, Nutrients & Obesity: Impact on Inflammation & Adipose Tissue (1:30 PM-3:30 PM)

Title Rapid Activation of Lipogenic Genes and Lipid Accumulation in the Liver of C57Bl/6 Mice after High-Fat Diet Exposure

Author String SPD Senthil Kumar, EJ Miller, EG Spicer, H Shi
Miami University, Oxford, OH

Body Obesity is associated with adverse metabolic effects including type-2 diabetes and its related glucose intolerance and insulin resistance. Chronic exposure of the liver to elevated nonesterified free fatty acids (NEFA), associated with consumption of a high-fat diet (HFD), results in marked hepatic dysfunction and is hypothesized to be an important contributor to obesity-induced insulin resistance. Because hepatic insulin resistance occurs within a few days after exposure to a HFD, we first asked whether rapid changes in liver lipogenesis and lipid β oxidation might contribute to this development. Not all HFDs are same. HFDs high in poly- or mono-unsaturated fatty acid (PUFA or MUFA) are reputed to be less harmful and may be beneficial. Adult male C57Bl/6 mice were fed with either a low-fat diet (LFD; 12% fat), or one of the three HFDs with matched macronutrient contents (45% fat; MUFA, PUFA or SFA) for 16 weeks (long-term) or for 4 days (short-term). Triglyceride (TG) contents in the liver of SFA- and MUFA-fed mice were increased by five folds (16 weeks) and two folds (4 days) compared to those of PUFA- and LFD-fed mice, which is consistent with plasma very-low-density-lipoprotein (VLDL) and TG increase of the SFA- and MUFA-fed mice. These data suggest that PUFA protects mice from developing fatty liver. We measured the gene expressions of lipogenic transcription factors and enzymes (SREBP-1c and FAS) and enzymes for lipid metabolism and beta oxidation (ACC and PGC1 α) using quantitative PCR. The mRNA levels of SREBP-1c and FAS were increased in all three HFD-fed mice after 4-day HFD exposure, indicating rapid activation of lipogenesis in the liver. In contrast, ACC and PGC1 α were altered in PUFA- but not SFA- and MUFA- fed mice. These data suggest that the rapid activation of lipogenic genes and lipid accumulation in the liver after short-term exposure to HFDs constitutes an early change that may contribute to the early occurrence of hepatic insulin resistance, perhaps by increase lipogenesis and fatty acid flux into the hepatic portal circulation and thus leads to lipotoxic effects. Second, the protective effect of PUFA, but not MUFA, against the lipotoxic action of saturated fatty acids is due to reduced lipogenesis and increased beta oxidation. Exposure to PUFA, but not MUFA, decreases the risk of metabolic syndrome as compared to a SFA.

Sources of Research Support: NIH R15 DK090823-01(HS).

Nothing to Disclose: SPDSK, EJM, EGS, HS

Pub # P3-363

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diet, Nutrients & Obesity: Impact on Inflammation & Adipose Tissue (1:30 PM-3:30 PM)

Title Poly-, but Not Mono-, Unsaturated Fatty Acid Protects Against the Development of Obesity, Insulin Resistance, and Fatty Liver

Author String EG Spicer, SPD Senthil Kumar, EJ Miller, H Shi
Miami University, Oxford, OH

Body The incidence of obesity continues to increase worldwide, and evidence links this increase with the consumption of high-fat diets (HFD). Exposure to a HFD with saturated fatty acid (SFA) leads to increased body adiposity and insulin resistance. Not all HFDs are same. HFDs high in poly- or mono-unsaturated fatty acid (PUFA or MUFA) are reputed to be less harmful and may be beneficial. We hypothesize that feeding HFDs with different fatty acids leads to differential metabolic changes, such as adiposity and insulin resistance. Male C57Bl/6 mice were fed with either a low-fat diet (LFD; 12% fat), or one of the three HFDs with matched macronutrient contents (45% fat; MUFA, PUFA or SFA) for 16 weeks. SFA- and MUFA-fed mice, but not PUFA-fed mice, gained significantly more body weight and adiposity (measured by Echo-MRI) than LFD-fed mice did. All HFD groups had similar cumulative caloric intake over 16 weeks. These data suggest that SFA and MUFA, but not PUFA, promote lipid storage and obesity development. To evaluate their insulin sensitivity, plasma glucose and insulin levels were measured, the HOMA-IR was calculated, and ip glucose tolerance test and insulin sensitivity tests were performed. SFA- and MUFA-fed mice, but not PUFA-fed mice, had significantly higher levels of fasting and non-fasting insulin and glucose concentrations and HOMA-IR compared to LFD-fed mice. In addition, SFA- and MUFA-fed mice developed glucose intolerance with greater areas under the glucose curves, and insulin resistance with lower glucose decline rates, compared to LFD-fed mice, whereas PUFA-fed mice were as glucose tolerant and insulin sensitive as LFD-fed mice, suggesting that PUFA protects mice from developing insulin resistance. Circulating lipid profile was measured. Although FFA and high-density-lipoprotein (HDL) levels were not different among any groups, very-low-density-lipoprotein (VLDL) and triglyceride (TG) levels were significantly higher in SFA- and MUFA-fed mice compared to PUFA- and LFD-fed mice. Similarly, TG contents in the liver of SFA- and MUFA-fed mice were increased by five folds compared to those of PUFA- and LFD-fed mice, suggesting that PUFA protects mice from developing fatty liver. In conclusion, dietary fat from different sources have dissimilar metabolic effects. Exposure to a matched HFD that is high in PUFA, but not MUFA, is much less capable of driving significant body fat gain and decreases the risk of metabolic syndrome as compared to a SFA.

Sources of Research Support: NIH R15 DK090823-01 (HS).

Nothing to Disclose: EGS, SPDSK, EJM, HS

Pub # P3-364

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diet, Nutrients & Obesity: Impact on Inflammation & Adipose Tissue (1:30 PM-3:30 PM)

Title Initiation of a High-Fat Diet Increases Voluntary Exercise in Mice

Author String TS Tylee, BE Wisse
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Body **Background:** Voluntary activity energy expenditure (AEE) is a critical factor in the prevention and treatment of obesity. Humans show a reduced AEE following weight loss, dependent on relative hypoleptinemia. Rodents decrease AEE with chronic exposure to high-fat diet (HFD) and weight gain. However, little is known about whether hormonal regulators, such as leptin, contribute to the regulation of voluntary activity. We propose that AEE is physiologically regulated and in order to evaluate potential mechanisms of regulation, we initially determined the effect of HFD on wheel running (WR) in mice.

Hypothesis: Initiation of HFD will lead to increased voluntary exercise in an effort to compensate for increased caloric intake and initial weight gain.

Methods: Adult, male C57Bl/6 mice (n=16) were acclimated to metabolic cages with running wheels. WR distance was continuously recorded and reported in one minute bins. Baseline data was collected for 5 days on chow and 3 days following switch to HFD. Exercise patterns were evaluated to determine total WR distance, running time, running speed, bout duration and number of WR bouts.

Results: WR distance was significantly increased with HFD compared to baseline (chow 6139 counts vs. HFD 7993 counts, p-value 0.007), due entirely to increase in WR during the dark cycle. When compared to their own baseline, animals showed a 57±7% increase in WR distance after initiation of HFD (p-value <0.005). The increase in WR was due to increase in time spent running (chow 286 min vs. HFD 383 min, p-value <0.005). During the first 6 hours of the dark cycle, this increase in running time was due to increase in WR bout duration (chow 11.4 min vs. HFD 19.2 min, p-value 0.005). In the later 6 hours of the dark cycle, the increase in running time was due to increased bout frequency (chow 3.0 bouts/hr vs. HFD 4.2 bouts/hr, p-value <0.005). HFD did not alter running speed (chow 37.9 counts/min vs. HFD 39.5 counts/min, p-value 0.29).

Conclusion: The regulation of voluntary physical activity remains poorly understood. Our current study demonstrates that initiation of HFD leads to significant increases in voluntary exercise, altering both duration and frequency of exercise intervals at specific times during the dark cycle, which may represent regulation of exercise motivation, reward or endurance. Future studies will determine whether these changes result from, and are dependent on, an increase of hypothalamic leptin signaling following HFD onset.

Nothing to Disclose: TST, BEW

Pub # P3-365

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diet, Nutrients & Obesity: Impact on Inflammation & Adipose Tissue (1:30 PM-3:30 PM)

Title Availability of a High-Calorie Diet and Diet History Alter Food Intake and Reproductive Function in Socially Housed Female Rhesus Monkeys

Author String V Michopoulos, S Berga, ME Wilson
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Body Exposure to chronic psychosocial stress produces an array of highly comorbid adverse health consequences in women, including emotional eating, reproductive dysfunction, and metabolic syndrome. Consumption of high caloric diets (HCD) likely acts on reward pathways to provide comfort from the psychosocial stress but might also increase energy balance to alleviate stress-induced changes in metabolism and reproduction. Using social subordination in ovariectomized female rhesus monkeys as a model of psychosocial stress in women, we tested the hypothesis that subordinate females would consume significantly more calories from a HCD compared to dominant females and that this increase in caloric intake would diminish the hypersensitivity to estradiol (E2) negative feedback inhibition of luteinizing hormone (LH) characteristic of subordinate females. Food intake was quantified for 3 weeks during a no choice phase when only a low caloric diet (LCD) was available and during a choice condition when both a LCD and HCD were available. The order of diet presentation was counterbalanced across subject groups. At the start of the third week of each phase, E2 replacement therapy was initiated and LH levels quantified to assess E2 negative feedback efficacy. Availability of the HCD during the choice phase increased caloric intake and leptin levels in subordinate animals to levels greater than dominant animals, even though both dominant and subordinate animals preferred the HCD to the LCD. Consumption of the LCD during the LCD only phase was increased significantly only when this LCD only phase followed the choice condition. These effects of diet history were also evident on reproductive function, as the initial presentation of the HCD decreased baseline levels of LH as well as enhanced E2 negative feedback inhibition of LH secretion. Removal of the HCD also resulted in increased E2 inhibition of LH. Our data suggest that previous history of HCD exposure alters feeding behavior and reproductive function in a manner not dependent on its availability for at least 3 weeks following its removal. Further studies are necessary to determine the duration of these HCD exposure effects as well as to understand the mechanism by which they occur.

Sources of Research Support: NIH grants RO1HD46501 (MW), RR00165 and F31MH085445 (VM).

Nothing to Disclose: VM, SB, MEW

Pub # P3-366

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diet, Nutrients & Obesity: Impact on Inflammation & Adipose Tissue (1:30 PM-3:30 PM)

Title Role of Bile Acids in the Thermic Effect of Food: A Human *In Vivo* Study

Author String JA Gordon, JD Linderman, S Smith, RJ Brychta, AB Courville, KY Chen, FS Celi
Nartional Institutes of Heatth, Bethesda, MD; Nartional Institutes of Heatth, Bethesda, MD

Body
Background: Recent animal and *in vitro* studies indicate that bile acids (BA) act in an endocrine fashion via TGR-5, a G-protein coupled receptor present in brown adipose tissue and human skeletal muscle, to increase energy expenditure (EE) and may play an important role in the thermic effect of food. This cross-over study is aimed to assess the effects of endogenous and exogenous BA on EE in humans.
Methods: 20 healthy volunteers (11 M, 9 F, age 33.7± 11.9, BMI 23.9 ± 1.9), after two days of a weight-maintenance and macronutrient balanced diet (50% carbohydrate, 20% protein, 30% fat) underwent a 7-hour recording of EE in a respiration chamber for five consecutive days. For each day, after overnight fasts, one of the following interventions was performed one hour after the beginning of the recording: 1) 600 Kcal 100% carbohydrate liquid meal, 2) 600 Kcal 72% fat/8% protein/20% carbohydrate liquid meal, 3) CCK bolus (*Kinevac*, 0.04mcg/kg)/oral placebo, 4) oral placebo/iv placebo, 5) oral exogenous bile acids (*Ursodiol*, 15mg/kg)/iv placebo. Blood samples were drawn at 0', 30', 60', 90', 180', and 360'. The orders of procedures # 1 and # 2, and of procedures # 3 and # 4 were assigned in a double-blinded random fashion. Primary analysis: To compare EE during different interventions to that of the placebo day.
Results: Postprandial EE significantly ($p < 0.01$) increased, $7.0 \pm 2.2\%$ and $10.1 \pm 2.17\%$ for the carbohydrate and the fat interventions respectively when compared to placebo; however, there was no appreciable change with CCK or ursodiol ($-0.5 \pm 7.5\%$ $p = 0.45$ and $-1.4 \pm 7.8\%$ $p = 0.259$ respectively). The difference in EE observed between the carbohydrate versus the fat interventions did not reach statistical significance ($p = 0.07$)
A positive correlation between total BA versus EE ($r^2 0.2314$, $p < 0.04$) was observed following a carbohydrate meal, and a similar but not statistically significant trend ($r^2 0.06$, $p = n.s.$) was observed after a fat meal. On the other hand, under fasting conditions, no correlation between BA and EE was observed after the CCK or the ursodiol interventions.
Conclusions: Under fasting conditions, neither endogenous nor exogenous bile acids play a significant role in energy expenditure. However, our data suggest that BA may play a synergistic role together with caloric intake in contributing to individual differences in the thermic effect of food.

Sources of Research Support: Intramural Research Program of the NIDDK-NIH; ClinicalTrials.gov Identifier NCT00706381.

Nothing to Disclose: JAG, JDL, SS, RJB, ABC, KYC, FSC

Pub # P3-367

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diet, Nutrients & Obesity: Impact on Inflammation & Adipose Tissue (1:30 PM-3:30 PM)

Title Nrf2 Represses FGF21 during Long-Term High-Fat Diet-Induced Obesity in Mice

Author String G Sykiotis, D Chartoumbekis, P Ziros, A Psyrogiannis, V Kyriazopoulou, I Habeos
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Body Obesity is a multifactorial condition of metabolic deregulation commonly associated with high-fat diet (HFD) and characterized, among other aspects, by chronic oxidative stress. Fibroblast growth factor 21 (FGF21) has recently been identified as a novel hormone that regulates metabolism; its administration to HFD-induced obese mice had beneficial effects on carbohydrate and lipid metabolism. Nrf2 (NFE2-related factor 2) is a transcription factor that orchestrates the expression of a battery of antioxidant and detoxification genes under both basal and stress conditions. Nrf2 was recently shown to have additional important functions in long-term adaptations to metabolic conditions, including the control of lipid metabolism under both normal diet and HFD. The present study investigated the role of Nrf2 in a mouse model of long-term HFD-induced obesity and characterized its crosstalk to FGF21 in this process. Wild type (WT) and Nrf2 knockout (Nrf2-KO) mice were fed a HFD for 6 months. The Nrf2-KO mice were partially protected from HFD-induced obesity, having gained 25% less weight at the end of the HFD period. Accordingly, the Nrf2-KO mice also developed a less insulin resistant phenotype, as evidenced by significantly improved performance in i.p. glucose tolerance tests and i.p. insulin tolerance tests after the HFD period, with no differences before HFD initiation. Importantly, Nrf2-KO mice had significantly higher plasma FGF21 levels (2-fold, $p < 0.0001$) and significantly higher FGF21 mRNA levels in liver (2-fold, $p < 0.01$) and white adipose tissue (6-fold, $p < 0.01$) compared to WT. The expression of the FGF21-regulated genes PGC1alpha and PEPCK was also significantly elevated in the liver (approx. 20%, $p < 0.05$ for each gene) and white adipose tissue (approx. 90%, $p < 0.05$ for each gene). Thus, the favorable metabolic phenotype of Nrf2-KO mice under HFD was associated with higher expression and abundance of FGF21 and with activation of its downstream transcriptional program. Consistently, the overexpression of Nrf2 in cultured ST-2 cells, a cell line derived from bone marrow stroma, resulted in significantly decreased FGF21 mRNA levels (40%, $p < 0.05$) as well as in significantly suppressed activity of a FGF21 promoter luciferase reporter (40%, $p < 0.05$), indicating that Nrf2 represses FGF21 expression in a cell-autonomous manner. The identification of Nrf2 as a novel regulator of FGF21 expands our understanding of the crosstalk between metabolism and stress defense.

Nothing to Disclose: GS, DC, PZ, AP, VK, IH

Pub # P3-368

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diet, Nutrients & Obesity: Impact on Inflammation & Adipose Tissue (1:30 PM-3:30 PM)

Title *Klotho*-Deficient Mice Are Resistant to High-Fat Diet-Induced Obesity

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Body *Klotho* is a transmembrane protein, and shown to have an adipogenesis-promoting effect. Genetically ablating *klotho* function from mice resulted in generation of lean mice with barely detectable fat tissues in the body [1, 2]. To study whether absence of *klotho* can provoke resistance to high-fat diet-induced obesity, we examined the effects of high-fat diet in mice lacking *klotho* activity. We have provided wild-type mice and *klotho* knockout mice with high-fat (60%) diet, and compared them with normal-fat (20%)-fed counterparts. Wild-type mice fed with high-fat diet gradually gained body weight, compared to the normal-fat fed mice. The average body weight of wild-type mice received high-fat diet for 12 weeks was 30.8 ± 0.43 g, which was significantly ($p < 0.05$) higher than the wild-type mice that received normal-fat diet (26.2 ± 0.84 g) for similar duration. However, such high-fat diet-induced weight gain was not observed in *klotho* knockout mice. Moreover, a clear increase in hepatic fat cell accumulation was noted in the high-fat diet fed wild-type mice, while, no such fatty changes in the liver was observed in high-fat diet-fed *klotho* knockout mice. After 9 weeks of high-fat diet, the average liver weight of wild-type mice was 1.7 ± 0.22 g, which was significantly ($p < 0.05$) higher than their normal-fat fed counterparts (1.12 ± 0.05 g). After 9 weeks of high-fat diet, the average liver weight of *klotho* knockout mice was 0.54 ± 0.01 g, while, the liver weight of *klotho* knockout mice fed with normal-fat diet for similar period was 0.51 ± 0.03 g. Of relevance, the average 24 hour food intake for normal and high-fat diet, in ratio to body weight, was similar for either wild-type or *klotho* knockout mice. The results of our dietary manipulation studies suggest that *klotho* knockout mice are resistant to high-fat diet induced obesity and associated complications.

(1) Nakatani et al., FASEB J 2009; 23:433-41.

(2) Ohnishi et al., FASEB J 2010; 24:3562-71.

Nothing to Disclose: JA, MO, SK, KT, AA, MSR

Pub # P3-369

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diet, Nutrients & Obesity: Impact on Inflammation & Adipose Tissue (1:30 PM-3:30 PM)

Title Yoyo Dieting vs. Obesity: A Longitudinal Analysis of Body Composition, Glucose Metabolism and Lifespan in Mice Susceptible to Diet-Induced Obesity

Author String EO List, DE Berryman, J Wright-Piekarski, R Leone, L Kutz, K Funk, B Tysl, A Bornschein, JJ Kopchick
Ohio University, Athens, OH; Ohio University, Athens, OH; Universidade Federal do Parana, Curitiba, Brazil; Ohio University, Athens, OH

Body It has been suggested that fluctuating body weight is unhealthy to the point that remaining obese may be preferable to a fluctuating body weight (1). Unfortunately, reports in the literature have argued both sides of this important health issue, and there is no clear consensus on the topic. Furthermore, limitations are common in observational type studies on this topic and include failures to distinguish intentional vs unintentional weight loss and failures to distinguish the effects of weight cycling in obese as opposed to normal-weight individuals. Therefore, to investigate the effects of repeated intentional weight loss and gain on health and longevity, we placed C57BL/6J mice on one of three dietary regimes: a high fat (HF) diet, a low fat (LF) diet or a yoyo diet (4 weeks HF, 4 weeks LF). The diets were maintained the entire lifespan of the mice. Body weight, food consumption, body composition, fasting blood glucose, glucose tolerance and longevity were determined.

As expected, food consumption, body weight, body fat, fasting blood glucose and glucose tolerance were significantly elevated in the HF mice compared to LF controls. Diet cycling resulted in increases in these same parameters during the HF portions of the yoyo diet. During the LF portions of the yoyo diet, all parameters were decreased. Thus, mice on the yoyo diet continuously cycled between a relatively obese and diabetic state followed by a relatively lean and non-diabetic state. Analysis of body composition revealed that the increases and decreases in body weight were due to changes in fat mass. Longevity data showed that the yoyo diet group lived significantly longer than the HF group of mice with average lifespans of 2.04 ± 0.19 and 1.55 ± 0.19 years, respectively. Most surprisingly, mice on the yoyo diet had a similar lifespan to that of the LF fed group with an average lifespan of 2.09 ± 0.28 years.

Based on our results in mice, it appears that yoyo dieting has a much better effect on long term health and lifespan than remaining obese. Furthermore, while several health parameters were negatively impacted during the HF cycle of the yoyo diet, little if any negative effect on longevity was observed. While remaining on a stable healthy diet provides the best outcome for health and longevity, it appears that it is much better to attempt to lose weight even in the face of repeated failures and fluctuating weight than to remain obese.

Rzehak P et al., Eur J Epidemiol 2007;22:665

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Nothing to Disclose: EOL, DEB, JW-P, RL, LK, KF, BT, AB, JJK

Pub # P3-370

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diet, Nutrients & Obesity: Impact on Inflammation & Adipose Tissue (1:30 PM-3:30 PM)

Title The Combination of Stress, Antidepressant Use, and High-Fat Diet Is a Risk Factor for Chronic Obesity

Author String C Mastronardi, G Paz-Filho, J Licinio, M-L Wong
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Body Obesity and major depression (MD) are major public health problems worldwide. Over 60% of Americans are overweight or obese; 16% of the population has a lifetime diagnosis of MD. These disorders have a combined economic burden of over \$150 billion/year. Approximately 27 million Americans are currently prescribed antidepressants (ADP). ADP dispensing increased substantially in the last two decades in parallel with a marked increase in obesity rates. We have therefore hypothesized a cause and effect relationship between these two disorders. Integration of animal and clinical studies has been a challenge due to contrasting outcomes: rodents tend to lose weight on antidepressants; in contrast, humans can gain weight. Our studies were designed to bridge this gap between animal and clinical data. We believe that such discrepancies occurred because previous animal studies did not appropriately model the environmental and dietary context of human ADP treatment. To address those shortcomings, we created a novel animal paradigm consisting of (i) exposure to stress, (ii) short-term behaviorally effective ADP treatment, (iii) followed by 6 months of high fat diet. We studied adult male Sprague-Dawley rats, housed at 24[thinsp][deg]C and 12[thinsp]h light/dark schedule (lights on from 0600 to 1800[thinsp]h) in a pathogen-free environment. Our results show that repeated restraint stress (RRS) rapidly and acutely decreased body weight gain (cumulative 1486±6[thinsp]g x d for RSS and 1592±14[thinsp]g x d for non-RSS, $P<0.0001$), that was gradually regained in young-adult rats fed on a high-fat diet. Interestingly, short-term ADP (fluoxetine or imipramine) treatment during RRS was followed by increased caloric intake late in the post-stress recovery period that resulted in larger weight and body size, long after ADP treatment had been discontinued (498±3[thinsp]g for RSS-fluoxetine and 477±3[thinsp]g for RSS-no antidepressant, $P<0.001$). Long-term outcomes of ADP treatment on body weight became evident during environmental conditions that combined high stress and chronic high fat intake. Our results support the concept that short-term ADP use might suffice to cause an insidious increase in caloric intake and body weight gain in the context of environment-pharmacological interactions. We propose that the combination of exposure to antidepressants, stress, and high-fat diet might be an insidious and overlooked, but important, contributor to the current obesity epidemic.

Sources of Research Support: Australian National University Institutional Funds.

Nothing to Disclose: CM, GP-F, JL, M-LW

Pub # P3-371

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diet, Nutrients & Obesity: Impact on Inflammation & Adipose Tissue (1:30 PM-3:30 PM)

Title Effect of High-Fat Diet on Glucose Metabolism in Selenocysteine Lyase Knockout Mouse

Author String LA Seale, AC Hashimoto, CL Gilman, A Seyedali, MJ Berry
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Body Dietary selenium is incorporated into selenoproteins cotranslationally as selenocysteine (Sec), the 21st amino acid. Sec is encoded by UGA codons, which typically function as stop codons. This process involves recoding of selenoprotein mRNAs via specialized secondary structures that recruit selenoprotein-specific trans-acting factors. Sec is unique in that it is synthesized on its cognate tRNA from a serine precursor, and the amino acid cannot be directly reutilized following protein degradation. Sec needs to be broken down to selenide in order to be reused by the Sec biosynthesis machinery. The Sec recycling reaction is catalyzed by Sec lyase (SLY), and produces alanine and selenide, the latter of which is readily available for selenophosphate synthetases (SPSs) where it can reenter the Sec biosynthesis cycle. We obtained SLY knockout mice from the Knockout Mouse Project (KOMP), a consortium with the University of California at Davis. The animals are viable, fertile and show no apparent phenotype when fed normal chow. However, when fed a high-fat diet (45% lipid content) for 10 weeks these mice have an increased body weight when compared to their wild-type counterparts. They tend to accumulate more fat in their white gonadal depots (gWAT) and in the interscapular brown fat (iBAT). However, although fatter, they have normal fasting glucose compared to pre-diabetic wild-type animals, suggestive of a delayed disease outcome. A gWAT and iBAT selenoprotein and metabolic map was generated and our results indicate the importance of the Se recycling pathway in glucose and fat homeostasis of rodents.

Sources of Research Support: NIH Grant R01DK047320 awarded to MJB.

Nothing to Disclose: LAS, ACH, CLG, AS, MJB

Pub # P3-372

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diet, Nutrients & Obesity: Impact on Inflammation & Adipose Tissue (1:30 PM-3:30 PM)

Title A Comparison of Metabolic Characteristics among C57BL/6NTAC, C57BL/6J and C57BL/6JBOM Diet-Induced Obese Mice with Environmental Conditioning

Author String MD Hayward, T Chu, S Karagrigoriou, D Chen, W Campbell, C Mottershead, A Wozniczka, G Bothe, JM Phelan, DS Grass, O Buiakova
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Body The C57BL/6 mice are extensively used in research on metabolism and obesity for several reasons, not least of which is the susceptibility of this strain to obesity, hyperglycemia and insulin resistance when fed a high fat diet (HFD): the [ldquo]diet induced obese[rdquo] (DIO) mouse model. Since there are multiple substrains of C57BL/6 mice that have identified genetic differences, we undertook a phenotypic analysis of metabolic characteristics in three substrains of C57BL/6: C57BL/6NTac, C57BL/6J and C57BL/6JBom on a regular, low fat diet and a HFD. Environment can have a significant influence on feeding and metabolic activity, so we further subdivided the strains fed a HFD into two conditioning groups, those fed a HFD beginning at 6 weeks of age at one of two commercial facilities: Taconic Inc, Germantown, NY (Tac) or The Jackson Laboratory, Bar Harbor, ME (Jax) and those fed a HFD beginning at 6 weeks of age at the same facility where the testing occurred, Taconic in Cranbury, NJ (TC). In general, the DIO mice from the B6NTac substrain were heavier than the B6J substrain at matched ages and conditioning sites. Additionally, the DIO strains that were fed the HFD at TC were heavier than the strains that were fed a HFD at the respective commercial suppliers (Tac or Jax), though the relative differences in body weight between the B6NTac and B6J substrains were consistent, regardless of the HFD conditioning location. The B6JBom substrain, which is not commercially available as a DIO model, was similar in weight to the B6J when both were conditioned to the HFD at TC. There was no difference in body weights among the B6NTac, B6J and B6JBom on the regular diet. The differences in weight among the HFD fed mice could be attributed to increased adiposity, based on DEXA results and weights of dissected adipose depots. Important differences in glucohomeostasis were noted among the different DIO substrains. The two DIO strains from Tac and Jax were similar in glucose levels during an OGTT assay at 18 weeks of age but insulin levels were much higher in the Tac mice, consistent with increased insulin content in the pancreas. Insulin resistance was greatest in both B6NTac DIO groups (conditioned at Tac and TC) when tested in an insulin tolerance test at 21 weeks of age. In summary, it appeared that the B6NTac substrain develops obesity and insulin resistance to a greater extent than the diet- and age-matched B6J and B6JBom mice.

Disclosures: MDH: Employee, Taconic. TC: Employee, Taconic. SK: Employer, Taconic. DC: Employee, Taconic. WC: Employee, Taconic. CM: Employee, Taconic. AW: Employee, Taconic. GB: Founder, Taconic JMP: Employee, Taconic. DSG: Employee, Taconic. OB: Employee, Taconic.

Pub # P3-373

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diet, Nutrients & Obesity: Impact on Inflammation & Adipose Tissue (1:30 PM-3:30 PM)

Title Macrophage-Conditioned Medium Impairs the Differentiation-Associated Reduction in Fibronectin and Collagen I/III Expression in Human Preadipocytes

Author String A Gagnon, MN Yarmo, A Landry, A Sorisky
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Body The adipose tissue of obese individuals is characterized by increased fibrosis, which has been attributed to immune cell infiltration. The extracellular matrix (ECM) plays a key role in the regulation of adipogenesis. Conversely, adipogenesis is associated with extensive changes in ECM composition. We, and others, have shown that macrophage-derived factors inhibit adipogenesis. The studies described here examined the effect of macrophage-conditioned medium (MacCM) on the expression of matrix proteins, fibrillar collagens I/III and fibronectin, in preadipocytes under control and adipogenic conditions. THP-1-monocytes were differentiated into macrophages with 100nM PMA for 24h and then placed in medium for another 24h (THP-1-MacCM) after removal of PMA. Medium conditioned by monocytes (THP-1-MonCM) and control medium (not conditioned by cells), were generated in parallel. Human preadipocytes were isolated from subcutaneous abdominal adipose tissue, grown to confluence, and then placed in control medium, THP-1-MonCM, or THP-1-MacCM with or without adipogenic inducers for 14 days. Adipogenesis in control medium reduced collagen I/III and fibronectin protein levels (by 51% and 92%, respectively (vs preadipocytes, $p < 0.05$, $n = 3$). When adipogenic inducers were added to THP-1-MacCM, adipogenesis was inhibited, and the reduction of fibronectin expression was attenuated (48% decrease vs preadipocytes, $p < 0.05$, $n = 3$), with no decrease in collagen I/III levels. When preadipocytes were treated for 14 days with THP-1-MacCM vs control medium without adipogenic stimuli, collagen I/III and fibronectin levels rose by 1.4 and 1.7 fold, respectively ($p < 0.05$, $n = 3$). THP-1-MonCM had no effect on adipogenic induction or collagen I/III and fibronectin regulation. Conditioned medium generated from LPS-activated human monocyte-derived macrophages, also inhibited adipogenesis of human preadipocytes, and attenuated the differentiation-dependent reduction in fibronectin expression. These effects were not observed with medium conditioned by vehicle-treated monocyte-derived macrophages. Our results suggest that macrophage-derived factors can regulate the expression of extracellular matrix proteins in preadipocytes. Further studies are needed to investigate if these changes in the extracellular environment contribute to the anti-adipogenic action of MacCM.

Sources of Research Support: Heart and Stroke Foundation of Ontario Grant NA6634 awarded to AS; Heart and Stroke Foundation of Ontario Master's Studentship awarded to MNY.

Nothing to Disclose: AG, MNY, AL, AS

Pub # P3-374

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diet, Nutrients & Obesity: Impact on Inflammation & Adipose Tissue (1:30 PM-3:30 PM)

Title Pro-Survival Activity of Macrophage-Conditioned Medium on 3T3-L1 Preadipocytes Is Impaired When Macrophages Are Activated by LPS

Author String ASD Molgat, A Gagnon, A Sorisky
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Body Obesity is associated with macrophage accumulation in the adipose tissue of mice and humans. We have previously reported that macrophage-conditioned medium (MacCM) prevents preadipocyte apoptosis in a PDGF-dependent manner. Adipose tissue macrophages have been reported to exist in various states of activation. Here, we have investigated the effect of pro-inflammatory macrophage activation on the pro-survival activity of MacCM and on MacCM-stimulated preadipocyte PDGFR signaling. MacCM was generated by treating J774A.1 macrophages with 100ng/ml lipopolysaccharide (LPS) or vehicle control for 0.5-6h, and then placing them in serum-free medium for 24h. Preadipocytes (3T3-L1) were placed in serum-free control medium, MacCM (vehicle control), or LPS-activated MacCM, and their survival was assessed. Preadipocyte cell number decreased by 48% upon serum-withdrawal ($p < 0.001$, 6h control medium without vs with serum); MacCM completely prevented this cell loss. LPS activation of macrophages impaired the pro-survival effect of MacCM, resulting in a 25% reduction in cell number ($p < 0.05$, 6h LPS- vs vehicle-treated MacCM). The attenuation of pro-survival activity observed with LPS-treated MacCM was not associated with a reduction in pro-survival PDGF signaling. We have previously reported that PDGFR, Akt and ERK phosphorylation are necessary for MacCM-dependent preadipocyte survival, and these three responses were the same for both vehicle- and LPS-treated MacCM. Untreated J774A.1 macrophages expressed PDGFA and PDGFB mRNA; PDGFB message was 14-fold more abundant. A transient 2-fold upregulation in macrophage PDGFB expression was observed 2h post LPS treatment, whereas PDGFA expression was reduced by 50% following LPS treatment. Our data suggest that macrophage activation with LPS significantly interferes with the pro-survival activity of MacCM, without altering MacCM-stimulated PDGFR-signaling. Further studies will be needed to identify the mechanism by which LPS-activation hinders the pro-survival activity of MacCM.

Sources of Research Support: Heart and Stroke Foundation of Ontario Grant NA6634 awarded to AS; Alexander Graham Bell Canada Graduate Scholarship from the National Sciences and Engineering Research Council of Canada awarded to ASDM.

Nothing to Disclose: ASDM, AG, AS

Pub # P3-375

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diet, Nutrients & Obesity: Impact on Inflammation & Adipose Tissue (1:30 PM-3:30 PM)

Title Human Adipose Tissue: Kinetics of Inflammatory and Angiogenic Factor Production during Weight Loss

Author String E Dalmas, C Poitou, J Aron-Wisniewsky, K Clement
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Body **Introduction:** Obesity is characterized by a systemic low-grade inflammation, partially reversible with weight reduction. Adipose tissue (WAT) is considered a site of production of inflammation-related factors in obesity. The whole spectrum of WAT secretion in obesity and its variations during fat mass loss is not fully established.

Methods: To address this question, we used Luminex-based proteomics to measure the secretion rate of 27 factors in 24 h media of subcutaneous WAT explants obtained in 23 obese patients (BMI: $48.8 \pm 1.7 \text{ kg/m}^2$) before and 3, 6 and 12 months after bariatric surgery. Adipocytes diameters and CD68+ macrophages content were determined in WAT slides obtained at the same time points.

Results: Kinetics profiles were highly variables depending on the factor secreted. In agreement with reduction in adipocyte diameter, leptin release dropped by 70 % at month 3 and slightly decreased thereafter, while adiponectin remained roughly stable during the whole time course of weight reduction. The secretion rate of the anti-inflammatory cytokines IL-10 and IL-13 increased up to 2-fold the initial values after 3 months and remained stable thereafter. Unexpectedly, a similar kinetic profile was observed for pro-inflammatory factors, including TNF α , IL-12 and RANTES. Beside, a series of other pro- or anti-inflammatory factors (IFN γ , IL-1 β , IL-1Ra, IL-4, MIP1 α , MIP1 β , IP-10) did not significantly change after bariatric surgery. By contrast, the rate of secretion of three major pro-angiogenic factors (bFGF, PDGF-BB and VEGF) increased regularly reaching 5 to 10-fold the pre-surgical values at month 12. Different patient's kinetic profiles were found for WAT CD68+ macrophages, with increased, stable or decreased counts. Early variations in WAT macrophage content between baseline and 3 months were positively correlated with changes in the release of pro-inflammatory factors in paired explants.

Conclusion: Weight loss induces distinct profiles of secretion in WAT, which are not attributable to the pro- or anti-inflammatory activity of the secreted factors. A regular and marked up-regulation of a number of pro-angiogenic factors suggests local stimulation of angiogenesis during the course of weight reduction. Change in WAT macrophage phenotype is under study to further explore the contribution of these immune cells to the dynamics of WAT secretion, tissue remodeling and related bioclinical phenotypes.

Nothing to Disclose: ED, CP, JA-W, KC

Pub # P3-376

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diet, Nutrients & Obesity: Impact on Inflammation & Adipose Tissue (1:30 PM-3:30 PM)

Title Markers of Macrophage Infiltration and Measures of Lipolysis in Human Abdominal Adipose Tissues

Author String M Pelletier, A Michaud, S Noel, PY Laberge, A Tcherno

Body Laval University Medical Center, Quebec City, Canada; Laval University, Quebec City, Canada; Laval University Medical Center, Quebec City, Canada

Obesity is associated with chronic, low-grade inflammation and macrophage infiltration in adipose tissues. Recent data suggested that local lipid fluxes and especially lipolysis are central regulators of adipose tissue macrophage (ATM) recruitment. **Objective:** We tested the hypothesis that high lipolytic rates are related to increased expression of ATM genes in human adipose tissues. **Methods:** Omental (OM) and subcutaneous (SC) fat samples were obtained by surgery in 46 women (age: 47.2±4.7 years, BMI: 26.9±5.2 kg/m²). Body composition and fat distribution were measured by dual energy x-ray absorptiometry and computed tomography. Mature adipocytes were isolated by collagenase digestion, lipolysis was measured by glycerol release in basal-, isoproterenol (10⁻⁵M)- and forskolin (10⁻⁵M)-stimulated conditions and data were expressed as fold-over-basal. Quantification of ATM gene mRNA expression (CD11b and CD68) was performed by RT PCR. **Results:** SC CD68 mRNA abundance was positively associated with BMI (r=0.29, p[le]0.05) and SC adipocyte size (r=0.36, p<0.05). In the same depot, CD68 mRNA was positively correlated with isoproterenol-stimulated lipolysis (r=0.36, p<0.05). This association remained significant after adjustment for total body fat mass (r=0.34, p<0.05) or SC adipocyte size (r=0.38, p[le]0.01). A similar association was found with forskolin-stimulated lipolysis in the SC depot (r=0.30, p<0.05), but it was not significant after adjustment for body fat mass or adipocyte size. In the OM depot, CD11b mRNA abundance was positively associated with BMI (r=0.34, p<0.05). CD11b mRNA expression level also correlated with isoproterenol-stimulated lipolysis (r=0.42, p[le]0.005) in the OM depot. This association remained significant after adjustment for total body fat mass and adipocyte size (r=0.41 and r=0.43, p<0.01 for both). CD11b mRNA level was also correlated with forskolin-stimulated lipolysis in the OM depot (r=0.37) and this association remained significant after adjustment for total body fat mass (r=0.33) or adipocyte size (r=0.33, p<0.05 for all). In subgroup analyses, high lipolytic rates in SC adipocytes were related to increased expression of CD68 in this compartment (p[le]0.001). High lipolytic rates in OM adipocytes were related to increased OM expression of CD11b (p<0.05). **Conclusion:** A high lipolytic rate in human adipose tissue is related to increased expression of ATM markers in the corresponding compartment, independently of adiposity and fat cell size.

Nothing to Disclose: MP, AM, SN, PYL, AT

Pub # P3-377

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diet, Nutrients & Obesity: Impact on Inflammation & Adipose Tissue (1:30 PM-3:30 PM)

Title Blunted Vascular Pi3-Kinase Signaling in a Novel Porcine Model of Diet-Induced Obesity Is Due to Increased Total P85alpha and Is Accompanied by Vascular Dysfunction

Author String CCL Wang, Y Xu, JW Leitner, L Lu, B Draznin, C Greyson, JEB Reusch, GG Schwartz
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Body Vascular dysfunction in insulin resistance is not well understood. We developed a novel porcine model of diet-induced obesity that manifests systemic insulin resistance and vascular insulin signaling defects. We hypothesized that the blunted vascular insulin signaling was due to increased vascular p85 alpha regulatory subunit, which could compete with functional p85-p110. We induced obesity in Yucatan micropigs (n=16) using a diet high in fat and simple sugars (HFHS). A lean control group (C, n=16) was fed standard pig chow. Baseline and 6 month IV glucose tolerance testing (IVGTT) and flow-mediated vasodilation (FMD) were performed. After 6 months on assigned diet, half of each group was given insulin 10 u/kg IV while half was not. Ten minutes later, pigs were euthanized and thoracic aortas excised to examine insulin receptor substrate-1-associated phosphatidylinositol 3-kinase (PI3K) activity, Akt phosphorylation, and total p85alpha expression. At the end of the study, pigs on HFHS diet were obese compared with those on C diet (82±9 kg vs. 37±5 kg, p<0.0001). Fasting glucose, triglycerides, and systolic blood pressure were also significantly higher on the HFHS diet. The area under the curve for glucose (AUCglu) in the IVGTT did not differ between groups, but AUCins was higher on the HFHS diet, consistent with systemic insulin resistance. PI3K activity was similar between groups in the absence of insulin, but markedly decreased in aortic lysates stimulated with insulin in vivo in HFHS vs. C (p<0.05). Total p85alpha was increased in aortic lysates from HFHS group compared with C ([uarr]37%, p<0.01). Isoform non-specific phospho-Akt/total Akt did not differ between groups, but insulin-stimulated phosphorylation of individual Akt isoforms phospho-Akt1 and phospho-Akt2 was 50% and 21% lower in HFHS compared with C (each p<0.05), while phospho-Akt3 was similar between groups. FMD was markedly decreased on HFHS compared with C (2±3% vs. 13±3%, p=0.02). These data demonstrate that impairment in systemic insulin signaling is accompanied by blunting of insulin-stimulated IRS-1-associated PI3K activity in the aorta, and that the defect in PI3K activity is due at least in part to increased total p85alpha. Insulin-stimulated isoform-nonspecific Akt phosphorylation is not affected, but phosphorylation of Akt1 and Akt2 are decreased. These abnormalities may contribute to vascular dysfunction found in this model of diet-induced obesity and insulin resistance.

Sources of Research Support: Veterans Affairs Office of Research and Development Career Development Award (CCLW); NIH/NHLBI.

Nothing to Disclose: CCLW, YX, JWL, LL, BD, CG, JEBR, GGS

Pub # P3-378

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diet, Nutrients & Obesity: Impact on Inflammation & Adipose Tissue (1:30 PM-3:30 PM)

Title Antioxidants Manganese Superoxide Dismutase (MnSOD), Catalase (CAT), and Vascular Endothelial Growth Factor-A (VEGF-A) Ameliorate High Glucose (HG) Concentration-Induced Adipogenesis in Subcutaneous Adipose Tissue (scWAT)-Derived Human Mesenchymal S

Author String S Sen, D Alers, M Young, Y-C Kim, N Yadava, JE Silva
Baystate Medical Center, Springfield, MA; University of Massachusetts, Amherst, MA; University of Massachusetts, Amherst, MA

Body Background: Primary hMSCs obtained by flow cytometry (FACS) from stromal vascular fraction of scWAT can differentiate into mature adipocytes, osteocytes or chondrocytes. Unlike bone marrow (BM) derived MSCs, their differentiation is more susceptible to cell milieu changes. We examined gene expression (qRT-PCR) of hscMSCs exposed to HG. Adipogenic differentiation was confirmed by Oil Red O stain and osteocyte differentiation by Von Kossa. Based on our BM-MSc results, we postulated that reactive oxygen species (ROS) production secondary to HG exposure may be the cause of poor survival and increased adipogenesis in hscMSCs. Thus, intra-cellular antioxidants could counteract the adverse differentiation potential of HG milieu. Methods: We transduced hMSCs with Adenovirus containing MnSOD or CAT at 100 MOI before exposing to HG. Ad-eGFP (green fluorescent protein) was used as control vector. We also examined the effect of over-expression of VEGF-A, a vascular factor, inducible by Hypoxia-Inducible Factor 1 and possibly ROS. VEGF-A is also known to modify PGC1A and UCP1 gene expression which may accelerate MSC mitochondrial respiration and help reduce ROS. ROS generation was analyzed by FACS using DCF-DA stain and apoptosis by propidium iodide -annexin V dye incorporation. Results: HG increased hscMSC lipid accumulation by 3.5 fold, induced ROS production intra-cellularly and promoted apoptosis. HG increased adipogenic gene expression, Leptin (LEP, 10-fold), Perilipin (PLIN; 4-fold) and PPAR γ (16 fold) while it reduced bone formation markers Alkaline Phosphatase (ALPL, 4 fold) and osteocalcin (BGLAP-1.6 fold) mRNAs. MSCs transduced with Ad-MnSOD, CAT or VEGF-A improved MSC survival in HG by 1.6, 1.3 and 1.8 fold, respectively. MnSOD and to a lesser extent CAT over-expression, reduced adipogenic markers, while both rescued suppressed bone markers. VEGF-A reduced adipogenesis to a lesser extent than MnSOD or CAT and had minimal effect on ALPL. Conclusion: Over-expression of MnSOD, CAT and VEGF-A help to maintain pluripotency of hscMSCs in HG (MnSOD>CAT>VEGF-A). Correlation between HG-induced adipogenesis and ROS most likely results from excess oxidizable substrate delivery to mitochondria and our findings emphasize the protective role of intracellular anti-oxidants, particularly, mitochondrial MnSOD. VEGF-A, despite of its ROS reduction potential was less effective indicating HG in this case overwhelmed the oxidative capacity leading to the generation of ROS.

- (1) Folgiero V et al., Cell Transplant. 2010;19(10):1225-35
- (2) Asano A et al., Mol Cell Endocrin. 2001 Mar 28;174(1-2):71-6
- (3) Arany Z et al., Nature. 2008 Feb 21;451(7181):1008-12.

Sources of Research Support: Collaborative Biomedical Research Grant, a scientific collaboration between Baystate Medical Center and University of Massachusetts, Amherst.

Nothing to Disclose: SS, DA, MY, Y-CK, NY, JES

Pub # P3-379

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diet, Nutrients & Obesity: Impact on Inflammation & Adipose Tissue (1:30 PM-3:30 PM)

Title The Nicotinic Acetylcholine Receptor $\alpha 7$ ($\alpha 7nAChR$), Main Regulator of the Cholinergic Anti-Inflammatory Reflex, Is Expressed in Subcutaneous Mature Adipocytes and Is Down-Regulated in Human Obesity

Author String R Canello, A Zulian, C Locarno, A Della Barba, C Invitti, A Liuzzi, AM Di Blasio
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Body The cholinergic anti-inflammatory reflex regulates inflammation in peripheral tissues. Nicotinic Acetylcholine receptors (nAChrs) are the central mediators of this anti-inflammatory pathway and it is well known that also non-neuronal cells express functional nAChrs. A role of $\alpha 7nAChR$ on insulin sensitivity improvement has been already demonstrated in rat adipocytes. At present, no data are available on $\alpha 7nAChR$ expression in human adipocytes. In this study, we investigated expression of $\alpha 7nAChR$ in human visceral (VAT) and subcutaneous adipose tissue (SAT) and in fully mature adipocytes isolated from the latter depot. A total of 34 adipose tissue biopsies from normal weight and obese subjects were used to assess $\alpha 7nAChR$ expression levels by quantitative real time PCR (RTqPCR). We also evaluated $\alpha 7nAChR$ expression in metabolically healthy obese (H-OB) and diabetic (type 2 diabetes) obese patients (T2D-OB) SAT. Alpha7nAChR expression was significantly lower in SAT of obese compared to normal weight subject. Moreover, SAT from H-OB (n=8) and T2D-OB (n=8) subjects displayed similar down-regulated $\alpha 7nAChR$ expression levels. In normal weight subjects, $\alpha 7nAChR$ was more expressed in mature adipocytes fraction than in whole SAT. In isolated mature adipocytes from morbidly obese subjects (BMI>40), $\alpha 7nAChR$ expression was 75% lower compared to adipocytes from normal weight subjects. In contrast, no differences were observed in $\alpha 7nAChR$ expression levels in VAT of obese and normal weight subjects. Alpha7nAChR down-regulation correlated with adiponectin gene expression levels (Rho=0.88, p=0.0076) in obese adipocytes. The down-regulation of $\alpha 7nAChR$ was also observed at protein levels in isolated adipocytes from SAT of obese subjects. These results provide evidence that $\alpha 7nAChR$ is preferentially expressed by mature adipocytes of the subcutaneous compartment and that expression and protein levels are significantly decreased in obesity. Moreover, this observed down-regulation seem to be independent of the glycaemic status. Although a variety of cellular mechanisms may modulate $\alpha 7nAChR$ functional activity, tyrosine phosphorylation has been shown to be the major pathway for a rapid effect on this receptor and a broad spectrum of protein tyrosine-kinase inhibitors, able to potentiate $\alpha 7nAChR$, are available. The possibility of $\alpha 7nAChR$ modulation opens new insights for the treatment of low grade inflammation linked to human obesity.

Nothing to Disclose: RC, AZ, CL, ADB, CI, AL, AMDB

Pub # P3-380

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diet, Nutrients & Obesity: Impact on Inflammation & Adipose Tissue (1:30 PM-3:30 PM)

Title Tumor Necrosis Factor- α Increases Protein Tyrosine Phosphatase 1B Expression Via NF[κ]B Signaling Pathway in Rat Hypothalamic Organotypic Cultures

Author String Y Ito, R Banno, S Hagimoto, Y Ozawa, H Arima, Y Oiso
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Body Protein Tyrosine Phosphatase 1B (PTP1B) has been implicated as an important regulator of energy homeostasis, whereas it has not been fully elucidated what regulates PTP1B expression in the hypothalamus, particularly under obese conditions. High-fat diet increases hypothalamic PTP1B expression levels accompanied by leptin resistance, and there are several lines of evidence suggesting that inflammation occurs in the hypothalamus during diet-induced obesity (DIO). In the present study, we investigated the mechanism by which tumor necrosis factor-alpha (TNF α) modulated hypothalamic PTP1B expression in rat hypothalamic organotypic cultures. Sprague-Dawley rats of 16 days old were killed by decapitation, and hypothalamic slice sectioned at 350 [μ]m thickness containing arcuate nucleus were cultured in the serum-free medium for 48 h, and then the medium was changed to a defined serum-free medium under different experimental conditions. To see the effects of TNF α on PTP1B mRNA, protein expression and p65 phosphorylation, slices were incubated with TNF α (25, 50, 100 ng/ml) for 6-24 h, and in order to assess if PTP1B expression is mediated through NF-kappa-B (NF[κ][Beta]) pathway, slices were incubated with TNF α (100 ng/ml), salicylate (10 [μ]M), NF[κ][Beta]-p65 inhibitory peptide (p65IP) (150[μ]M) or combinations of TNF α with salicylate or p65IP for 24 h. Incubation with TNF α showed that TNF α increased PTP1B mRNA, protein expression and p65 phosphorylation in a dose- and time- dependent manner. Incubation with salicylate or p65IP markedly suppressed TNF α -induced p65 phosphorylation as well as PTP1B expression to almost their basal levels, while incubation with salicylate or p65PI alone had no effect on the basal levels of either p65 phosphorylation or PTP1B expression. These results suggest that TNF α regulates PTP1B expression via NF [κ][Beta] signaling pathway in hypothalamic organotypic cultures, and up-regulated NF[κ][Beta] in the hypothalamus caused by increased TNF α during DIO may cause leptin resistance by increasing hypothalamic PTP1B levels.

Nothing to Disclose: YI, RB, SH, YO, HA, YO

Pub # P3-381

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diet, Nutrients & Obesity: Impact on Inflammation & Adipose Tissue (1:30 PM-3:30 PM)

Title The Role of Visfatin in Palmitate-Induced Activation of Inflammatory Cytokines through Nuclear Factor- κ B in Hepatocytes

Author String YJ Choi, SE Choi, ES Ha, Y Kang, SJ Han, DJ Kim, Y-S Chung, KW Lee, HJ Kim
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Body
Objective: Free fatty acids (FFAs) lead to the activation of inflammatory pathways related to the induction of insulin resistance. Pre-B-cell colony-enhancing factor/nicotinamide phosphoribosyltransferase/visfatin (PBEF/NAMPT/visfatin) is known to play a role in obesity-related metabolic diseases and inflammatory conditions. Here, the role of PBEF/NAMPT/visfatin in FFA-induced inflammation was investigated in hepatocytes.
Methods: The following factors were examined: 1) the protein and mRNA expression of PBEF/NAMPT/visfatin in the liver tissue of insulin-resistant rats, 2) the expression of PBEF/NAMPT/visfatin in HepG2 cells treated with palmitate, 3) the palmitate-induced expression of interleukin (IL)-6 and tumor necrosis factor (TNF)- α in HepG2 cells transfected with PBEF/NAMPT/visfatin-specific siRNA, and 4) the expression of PBEF/NAMPT/visfatin in HepG2 cells treated with a nuclear factor κ B (NF- κ B) inhibitor (SN 50) and infected with Ad-I κ B α .
Results: The protein and mRNA levels of PBEF/NAMPT/visfatin were significantly higher in insulin-resistant rat liver tissue compared with control. PBEF/NAMPT/visfatin expression was significantly increased in HepG2 cells treated with palmitate, in a time- and concentration-dependent manner. PBEF/NAMPT/visfatin-specific siRNA significantly decreased the palmitate-induced expression of IL-6 and TNF- α . A NF- κ B inhibitor induced the down-regulation of PBEF/NAMPT/visfatin in HepG2 cells following treatment with palmitate. HepG2 cells infected with Ad-I κ B α showed decreased expression of PBEF/NAMPT/visfatin following treatment with palmitate.
Conclusion: The expression of PBEF/NAMPT/visfatin is closely associated with the expression of proinflammatory cytokines in FFA-induced inflammation and is significantly decreased by NF- κ B inhibition in HepG2 cells. PBEF/NAMPT/visfatin may play a role in FFA-induced inflammation in hepatocytes through the NF- κ B pathway.

Nothing to Disclose: YJC, SEC, ESH, YK, SJH, DJK, Y-SC, KWL, HJK

Pub # P3-382

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diet, Nutrients & Obesity: Impact on Inflammation & Adipose Tissue (1:30 PM-3:30 PM)

Title Early B-Cell Factor-1 (Ebf1) Regulates Nutrient Partitioning and Metabolism in Mature Adipocytes by Repressing Components of the PI3-K/Akt/mTOR Pathway While Stimulating Expression of Lipogenic Enzyme Genes

Author String MJ Griffin, ED Rosen
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Body Early B Cell Factor-1 (Ebf1) was originally identified as a transcription factor crucial for B-cell differentiation. Our lab and others have also shown that Ebf1 plays a crucial, albeit temporal, role in the early differentiation of preadipocytes and in the bone-fat switch. However, Ebf1 expression rises in the late stage of adipocyte differentiation, and remains elevated in mature cells; its function there is currently unknown. To determine the role of Ebf1 in mature adipocytes, we undertook a combination of microarray analysis of Ebf1-deficient 3T3-L1 cells as well as genome-wide Ebf1 ChIP-Seq. Our results indicate that many components of the PI3-K/Akt/mTOR pathway are repressed by Ebf1, including secreted factors such as Igf1, and this was accompanied by enhanced basal and insulin-stimulated Akt phosphorylation as well as increased basal and insulin-stimulated glucose uptake in Ebf1-knockdown cells. In addition, numerous genes normally expressed in osteoblasts (such as osteocalcin) were significantly up-regulated in Ebf1-deficient cells, suggesting that one function of Ebf1 in mature adipocytes is to repress the expression of bone-specific genes. Interestingly, the expression of lipogenic enzyme genes such as FAS and Scd1 was decreased in Ebf1-deficient cells, and this was accompanied by impaired insulin-stimulated lipogenesis, decoupling insulin signaling from lipid synthesis in this model. Our results suggest that Ebf1 functions as a major regulator of signal transduction and nutrient partitioning in mature fat cells.

Nothing to Disclose: MJG, EDR

Pub # P3-383

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diet, Nutrients & Obesity: Impact on Inflammation & Adipose Tissue (1:30 PM-3:30 PM)

Title Adipocyte Fatty Acid Binding Protein in Kupffer Cell Mediates Obesity-Related Non-Alcoholic Fatty Liver Disease through Endoplasmic Reticulum Stress and Inflammation

Author String KSL Lam, RLC Hoo, JYL Wong, A Xu
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Body **Introduction:** Circulating levels of adipocyte fatty acid binding protein (A-FABP) are elevated in nonalcoholic fatty liver disease (NAFLD) in humans and correlate with hepatic inflammation and fibrosis. Activated Kupffer cells (KC) are the major sources of elevated pro-inflammatory cytokines in NAFLD. We investigated whether KC was a major source of hepatic A-FABP expression leading to NAFLD and whether selective inhibitor of A-FABP can be used for the treatment of NAFLD.

Methods and Materials: (1) To study the role of KCs and A-FABP in acute liver injury, C57 mice, with or without KCs depletion induced by gadolinium chloride III, were intraperitoneally injected with lipopolysaccharide (LPS). (2) To study the role of A-FABP in obesity-related chronic liver injury, C57 mice were fed with high fat high cholesterol (HFHC) diet, with or without oral treatment with an A-FABP selective inhibitor, BMS309403 (BMS), for 16 weeks. Serum alanine aminotransferase (ALT), aspartate transaminase (AST) and hepatic fat content were measured by biochemical methods. Hepatic expression of A-FABP, pro-inflammatory cytokines, endoplasmic reticulum (ER) stress and fibrotic markers were assessed by quantitative-PCR and/or immunoblotting.

Results: Hepatic A-FABP mRNA and protein levels were increased by LPS injection or HFHC diet, accompanied by elevated ALT and AST levels and increased production of pro-inflammatory cytokines. Depletion of KCs reduced LPS-induced A-FABP expression in the liver, suggesting KCs as the major source of hepatic A-FABP. Oral administration with BMS alleviated glucose intolerance, insulin resistance and liver injury in HFHC-diet induced obese mice. H&E and oil red O staining indicated the alleviation of inflammatory status and steatosis in liver sections. These changes were accompanied by a markedly reduced hepatic expression of pro-inflammatory cytokines (TNF-alpha, MCP-1, IL-6), A-FABP, markers of fibrosis (pro-collagen and tissue inhibitor of metalloproteinases-1 [TIMP-1]) and ER stress (CCAAT/enhancer-binding protein homologous protein [CHOP], glucose regulated protein [GRP78] and X-box binding protein [XBP-1]).

Conclusion: Endotoxin and HFHC diet induced liver injury are associated with elevated A-FABP expression in KCs. A-FABP may play an etiological role in obesity-induced NAFLD by inducing ER stress and inflammation. The selective inhibitor of A-FABP may represent a promising drug candidate for treating NAFLD.

Sources of Research Support: RGC grant (GRF 768209) from the Hong Kong Research Grant Council.

Nothing to Disclose: KSL, RLCH, JYLW, AX

Pub # P3-384

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Diet, Nutrients & Obesity: Impact on Inflammation & Adipose Tissue (1:30 PM-3:30 PM)

Title Age Differences in Food-Intake and the Weight-Suppressant Effects of Self-Administered Nicotine

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Body

Introduction: Tobacco products are often used as a weight loss tool; however, nicotine cessation results in weight gain. This is a problem for adolescents who are particularly concerned about weight gain and are most vulnerable to tobacco abuse. Adolescent nicotine exposure enhances long-term vulnerability to tobacco abuse in adulthood. However, it is unclear whether the weight suppressant effects of nicotine are altered by adolescent nicotine exposure in an age-dependent manner. Thus, this study compared weight gain, food intake, and nicotine self-administration in rats from different stages of development and in adult rats that were previously exposed to nicotine during adolescence.

Methods: We used a model of nicotine self-administration (SA) whereby male rats were given 23-hour access to increasing doses of nicotine separated by brief periods of drug abstinence. All rats were first trained to perform operant responses for food and water in the SA chambers and were implanted with a jugular catheter for intravenous SA of nicotine. An additional group of adolescents were pretreated with nicotine (4.2 mg/kg/day) for 14 days and received catheters later in adulthood. Adult rats were given access to nicotine SA for 3, 4-day cycles whereby the dose of nicotine was increased between each cycle as follows: 0.03, 0.06, 0.09 mg/kg/0.1 ml infusion given over two cycles. Each cycle was separated by a forced period of abstinence for 3 days.

Results: Nicotine intake was highest in adolescents, intermediate in adults that were exposed to nicotine during adolescence, and lowest in drug-naïve adults. Adolescents displaying the highest food-intake and weight gain following chronic nicotine as compared to the other groups. Exposure to nicotine during adolescence blocked the food- and weight-suppressant effects of nicotine later in adulthood as compared to naïve adults that displayed robust weight suppressant effects of chronic nicotine.

Conclusions: There appears to be long-lasting metabolic consequences of adolescent nicotine exposure, including a reduction in the food-intake suppressant effects of this drug. These findings have clinical implications towards treating adolescent smokers with nicotine replacement therapy, as this approach may produce detrimental long-term consequences on food intake and weight control later in adulthood. Additionally, starting smoking at a young age, even with smoking cessation occurring may have detrimental long-term metabolic effects.

Nothing to Disclose: LAN, EE, MM, C-SS, OVT, TCF, LEO

Pub # P3-385

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Obesity, Inflammation & Diabetes (1:30 PM-3:30 PM)

Title A Novel Role for Lymphocytes in Non-Alcoholic Fatty Liver Disease Unmasked by High-Fat Diet-Induced Obesity in *RAG-1* Mice

Author String E Kodela, S Theocharis, C Kouskouti, K Kang, M Sleeman, K Karalis
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Body Obesity is characterized by a chronic, active, inflammatory process. Various studies have shown the significance of activated macrophages in the pathogenesis of obesity-associated insulin resistance and the metabolic syndrome. Recently, a critical role of lymphocytes in the biology of adipose tissue and the corresponding development of insulin resistance has been demonstrated. To further elucidate the role of lymphocytes in the pathogenesis of obesity and the metabolic syndrome, we compared the responses of the lymphocyte-deficient *rag1*^{-/-} and wild-type mice to high-fat diet (HFD) - induced obesity and liver steatosis. Following 8-10 weeks of HFD we found similar body weight changes in both genotypes. Glucose levels were similarly elevated in the two genotypes, while *rag1*^{-/-} mice exhibited increased insulin sensitivity, as assessed by the insulin tolerance test. Importantly, *rag1*^{-/-} mice showed no histological signs of liver steatosis in contrast to the significant infiltration detected in the wild-type mice. Proteomic and genomic analyses of liver tissue from both groups revealed significant differences in the expression of genes involved in lipid synthesis and oxidation, in agreement with the histological picture. Our preliminary data in wild-type HFD induced obesity obese mice, administered of CD1d antibody (0.5mg/mouse over 1 week period) that targets NKT cells, suggest a significant amelioration of their liver steatosis. Furthermore, similarly to the *rag1*^{-/-} mice, CD1d treated mice showed increased expression of fatty acid oxidation genes, decreased expression of pro-inflammatory genes and improved histological picture. These findings provide evidence for a critical role of lymphocytes in hepatic lipid metabolism and insulin sensitivity. The exact molecular factors mediating the above effects and their potential use in the development of novel therapeutic approaches for non-alcoholic fatty liver disease (NAFLD) in humans are under investigation.

Nothing to Disclose: EK, ST, CK, KK, MS, KK

Pub # P3-386

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Obesity, Inflammation & Diabetes (1:30 PM-3:30 PM)

Title Constitutive Activation of IKK β in Adipose Tissue Prevents Diet-Induced Obesity

Author String P Jiao, B Feng, J Ma, Y Nie, H Xu
Alpert Medical School of Brown University, Providence, RI; Jilin University, Changchun, China; Alpert Medical School of Brown University, Providence, RI

Body The I[κ]B kinase β (IKK β) is a master kinase known to involve in obesity-related inflammation and insulin resistance through NF- κ B-dependent and independent pathways. However, the effect of IKK β activation in adipose tissue, the organ critical for storage of excessive energy and initiation of inflammatory responses in the context of obesity, on systemic insulin sensitivity and metabolism has not been studied. In our study, we found that mice overexpressing the constitutively active IKK β in adipose tissue under the control of adipocyte protein 2 promoter (aP2-IKK β SE mice) were protected from age-related and diet-induced body weight gains, despite increased food intake. IKK β SE mice have significantly reduced weights in all white adipose tissue (WAT) depots, and reduced triglyceride contents in adipose tissue and liver. Despite increased systemic and adipose tissue inflammation, IKK β SE mice displayed decreased blood glucose levels, improved glucose and insulin tolerance. This may be attributable to a higher metabolic rate including increased oxygen consumption and energy expenditure in IKK β SE mice compared to control mice. Histological analysis revealed presence of many small adipocytes in gonadal WAT of IKK β SE mice fed on high fat diet. Furthermore, transgenic expression of IKK β in adipose tissue improved high fat diet-induced hepatosteatosis. Collectively, increased energy expenditure and reduced plasma free fatty acids (FFAs) may contribute to enhanced systemic insulin sensitivity in IKK β SE mice. Our study demonstrates that presence of inflammation in adipose tissue prior to the development of obesity has beneficial effect on metabolism through promoting energy expenditure.

Nothing to Disclose: PJ, BF, JM, YN, HX

Pub # P3-387

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Obesity, Inflammation & Diabetes (1:30 PM-3:30 PM)

Title Evidence of an Inflammatory State in a Mouse Model of Polycystic Ovary Syndrome

Author String JS Marino, AR Dowling, X Qiu, L Nedorezov, LFC Mueller, JW Hill
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Body Pro-opiomelanocortin (POMC) expressing neurons of the hypothalamus play an integral role in energy balance and glucose regulation. POMC neurons modulate metabolic function largely through central leptin and insulin receptor signaling. Indeed, mice lacking insulin and leptin receptor expression on POMC neurons develop obesity, insulin resistance, and infertility. The objective of this project was to test whether deletion of leptin and insulin receptors from POMC neurons leads to an altered lipid profile and a state of chronic inflammation. Serum was analyzed for triglycerides, total cholesterol, HDL, LDL/VLDL, C-Reactive Protein (CRP), and cytokines. Adipose tissue was labeled for macrophage antigen F4/80 to investigate the accumulation of macrophages. Real time PCR was performed to quantify the expression of genes associated with an inflammatory response in adipose, liver and skeletal muscle. We found that serum lipids and CRP were unaltered in female mice lacking leptin and insulin receptors on POMC neurons (POMCcre+IRLepR) compared to controls (POMCcre-IRLepR). While serum cytokine levels were not different between groups, adipose gene expression of IL-6 and IL-1 β were elevated in POMCcre+IRLepR mice compared to controls. Furthermore, POMCcre+IRLepR mice had a greater presence of F4/80 positive cells compared to controls. In support of histological findings, POMCcre+IRLepR mice had 2 fold higher mRNA levels of F4/80. While the liver of POMCcre+IRLepR mice had elevated IL-1 β and reduced insulin receptor gene expression, other cytokine and F4/80 mRNA levels were not different. Skeletal muscle showed no sign of a local inflammatory response. These preliminary experiments suggest that POMCcre+IRLepR female mice may suffer from a more pronounced adipose tissue inflammatory-like state compared to other peripheral tissues. Being that liver from POMCcre+IRLepR mice have lower insulin receptor gene expression, it is possible that over a longer period of time the liver may show a similar inflammatory phenotype.

Sources of Research Support: NIH Grant R00HD056491 awarded to JWH.

Nothing to Disclose: JSM, ARD, XQ, LN, LFCM, JWH

Pub # P3-388

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Obesity, Inflammation & Diabetes (1:30 PM-3:30 PM)

Title Cell Non-Autonomous Regulation of Aging and Mitochondrial Uncoupling by Insulin-Resistant Adipose Tissue

Author String SJ Russell, C Cederquist, JS Park, MA Mori, CR Kahn
Joslin Diabetes Center, Boston, MA

Body Lack of insulin signaling in the adipose tissue of fat-specific insulin receptor knockout (FIRKO) mice increases lifespan. To identify mechanisms of this cell non-autonomous regulation of aging, we assayed markers of aging, senescence, and oxidative stress in tissues of FIRKO and control mice. In control mice, expression of p16^{Ink4a} and p53 proteins, as well as p16^{Ink4a} mRNA, increased markedly in both adipose and non-adipose tissues as mice aged from 2 months to 2 years. These age-dependent increases in aging markers were attenuated in both adipose and non-adipose tissues of FIRKO mice. Furthermore, in individual control and FIRKO mice the levels of p16^{Ink4a} and p53 protein in different tissues were highly correlated, suggesting coordinated regulation of tissue aging. Activity of glutathione peroxidase (GPX), a critical component of cellular defense against oxidative stress, was reduced in multiple tissues of FIRKO mice relative to control mice, yet levels of lipid hydroperoxides were unchanged. Isolated liver mitochondria from FIRKO mice exhibited reduced GPX activity without increased lipid hydroperoxidation and higher basal oxygen consumption through an increase in uncoupled respiration. These results suggest that loss of insulin signaling in adipose tissue leads to cell non-autonomous induction of mitochondrial uncoupling, a generalized reduction of reactive oxygen species production, and promotion of longevity in FIRKO mice.

Nothing to Disclose: SJR, CC, JSP, MAM, CRK

Pub # P3-389

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Obesity, Inflammation & Diabetes (1:30 PM-3:30 PM)

Title Exenatide Exerts a Potent Anti-Inflammatory Action

Author String A Chaudhuri, H Ghanim, M Vora, K Korzeniewski, S Abuaysheh, A Makkissi, P Dandona
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Body In view of our previous report that exenatide induces a decrease in plasma C- reactive protein concentrations independently of weight loss, we have now tested the hypothesis that it may exert an anti-inflammatory effect in patients with type 2 diabetes. Twenty four patients were prospectively randomized to be injected subcutaneously with either exenatide 10 [mu]g daily (n=12, mean age: 53.8±4.2 years; mean BMI: 39.1±2.3kg/m²; mean HbA1c:8.2±0.4%) or placebo (n- 12, mean age: 55.6±3.3 years; mean BMI: 38.1±1.9kg/m²; mean HbA1c:8.6±0.4%) for 12 weeks. Fasting blood samples were obtained at 0, 3, 6 and 12 weeks. Blood glucose fell from 139±17 to 110±9mg/dl and HbA1c from 8.6±0.4 to 7.9±0.6% (P<0.05). There was a significant reduction in ROS generation, the mRNA expression of JNK-1 and TLR-4 in MNC by 18±8%, 20±11% and 16±7%, respectively (P<0.05 for all), and the plasma concentrations of MCP-1, MMP-9 and SAA by 18±8%, 20±11% and 16±7%, respectively (P<0.05 for all). In order to examine the possibility that these actions may be exerted acutely, we examined whether a single injection of exenatide would exert an acute anti-inflammatory effect. Blood samples were collected at 0, 2, 4 and 6h. The injection of exenatide resulted in a significant reduction of pro-inflammatory mediators, JNK-1 and TLR-4 at 2h by 15±4% and 23±7%, respectively (P<0.05 for all). This reduction was transient and coincided with the known pharmacokinetic peak of exenatide at 2h after an injection. We conclude that exenatide exerts an anti-inflammatory effect at the cellular and molecular level and that this may contribute to a potentially beneficial anti-atherogenic effect.

Sources of Research Support: NIH Grants R01-DK075877 and R01-DK069805 awarded to PD; American diabetes Grant awarded to PD; Investigator initiated grant from Amylin.

Disclosures: AC: Speaker, Eli Lilly & Company. PD: Clinical Researcher, Speaker, GlaxoSmithKline. Nothing to Disclose: HG, MV, KK, SA, AM

Pub # P3-390

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Obesity, Inflammation & Diabetes (1:30 PM-3:30 PM)

Title Combined Prednisolone Treatment and a High-Fat Diet in Mice Both Induce Hyperglycemia, but Alter Hepatic Glucose Metabolism Differentially

Author String AJ Laskewitz, TH van Dijk, WH Dokter, M-J van Lierop, A Grefhorst, F Kuipers, BK Groen
University Medical Center Groningen, Groningen, Netherlands; MSD, Oss, Netherlands; Erasmus Medical Center, Rotterdam, Netherlands

Body
Background: Sustained treatment with anti-inflammatory synthetic glucocorticoids can cause many severe side effects, e.g. weight gain, dyslipidemia and insulin resistance, all of which are also features of the metabolic syndrome. A major risk factor for the metabolic syndrome is use of a western style diet, containing a high amount of fat. The aim of this study was to investigate whether a high fat diet aggravates the effect of prednisolone treatment on insulin sensitivity and hepatic glucose metabolism.
Methods: C57Bl/6J mice were fed a high fat diet (36% from lard) for six weeks and treated with prednisolone (P) (10 mg/kg/d) or vehicle as a control (C) for the last 7 days by oral gavage. Mice were characterized for general metabolic parameters. Insulin resistance was assessed by fasting blood glucose kinetics and a hyperinsulinemic euglycemic clamp (HIEC). During the HIEC relevant fluxes in hepatic glucose metabolism were analyzed *in vivo* by employing mass isotopomer distribution analysis (MIDA).
Results: Sustained prednisolone treatment combined with a high fat diet elevated fasting blood glucose and plasma insulin concentrations compared to controls. This resulted in an increased HOMA-score (3.6 ± 1.3 vs. 7.3 ± 1.3 , C vs. P, $p < 0.05$). Fasting blood glucose kinetics showed that prednisolone treatment increased the endogenous glucose production (EGP), while feeding a high fat diet reduced the metabolic clearance rate (MCR), both suggestive for induced insulin resistance. Remarkably, the combination of a high fat diet and prednisolone treatment did not reduce insulin sensitivity, as evident from the glucose infusion rate (232 ± 25 vs. 253 ± 24 [μ]mol/kg/min, C vs. P, ns) during the HIEC. MIDA measurements showed an altered glycogen balance, which was caused by an increased glycogen phosphorylase flux. Since glycogen is an important source for energy during fasting we measured plasma fibroblast growth factor 21 (FGF21) levels, an important parameter for a fasting response. Plasma FGF21 was almost doubled by prednisolone, both in the fed and fasted state, which was confirmed by hepatic gene expression levels.
Conclusion: Treatment of mice with prednisolone and a high fat diet both induced hyperglycemia, but different pathways were involved. Interestingly, combined prednisolone and high fat diet treatment had no effect on insulin sensitivity as measured during the HIEC indicating that the hyperglycemia induced by the treatments is caused by insulin independent mechanisms.

Sources of Research Support: This work was performed within the framework of the Dutch Top Institute Pharma T1-106.

Nothing to Disclose: AJL, THvD, WHD, M-JvL, AG, FK, BKG

Pub # P3-391

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Obesity, Inflammation & Diabetes (1:30 PM-3:30 PM)

Title The Effect of Exogenous Erythropoietin on Lipid Metabolism and Inflammation in White Adipose Tissue of High-Fat Diet-Fed Mice

Author String R Meng, Y-P Wang, D-L Zhu
Nanjing Drum Tower Hospital, Nanjing University School of Medicine, Nanjing, China; Nanjing University School of Medicine, Nanjing, China

Body Erythropoietin (EPO), required for erythropoiesis via specific binding to its cell surface receptor (EPOR), was first used for clinic treatment in last century. Recently, it has been reported that EPO could ameliorate insulin resistance (IR) in clinic study, while the underlying mechanism remains obscure, which was investigated in the present study. We fed 4-week-old male C57BL/6 mice with high-fat diet (HFD, 60% kcal from fat) for 12 weeks to induce IR. Mice were then received injections of recombinant human EPO (1000U/kg, EPO group) or saline (HFD group) 3 times per week for 2 weeks. We found that weight gain in EPO group was significantly lower than that in HFD group from the 6th day to the end of the experiment without energy intake changes. Intraperitoneal glucose tolerance tests, fasting insulin and glucose showed that insulin sensitivity was significantly improved after EPO treatment. We also detected lipid metabolism status in the blood. The total cholesterol, HDL-c, LDL-c and FFA levels of HFD group were significantly elevated compared with the normal controls. EPO treatment completely reversed the detrimental effects of HFD feeding on total cholesterol, LDL-c and FFA levels ($p < 0.05$ resp.). The serum inflammatory factors were also detected. The level of IL-6 was remarkably lower in EPO group than that in HFD group. While TNF- α and leptin levels had a trend to decrease in EPO group as compared to HFD group. Importantly, EPOR was detected in white adipose tissue which indicated that EPO may have direct effects on white adipose tissue. We found that the diameter of adipocytes was significantly smaller in EPO group than that in HFD group ($p < 0.05$). Furthermore, sterol response element binding protein 1c, the expression of the transcription factors involved in lipogenesis, showed significant lower in EPO group than that in HFD group ($p < 0.05$). EPO group also exhibited remarkably higher lipoprotein lipase and lower fatty acid synthase mRNA expression compared to HFD control group ($p < 0.05$ resp.). Additionally, we found EPO treatment significantly decreased the expression of TNF- α , IL-6, MCP-1 and leptin ($p < 0.05$ vs. HFD group resp.) in EPO group. Taken together, our data indicated that exogenous EPO could attenuate dyslipidemia and inflammation induced by HFD both in the blood and white adipose tissue. IR was ameliorated by EPO through its multiple protective effects in HFD mice. Our findings may result in novel therapeutic strategies for IR and diabetes.

Sources of Research Support: National Natural Science Foundation of China 81070636; Natural Science Foundation of Jiangsu Province of China BS2006006, BS2010323.

Nothing to Disclose: RM, Y-PW, D-LZ

Pub # P3-392

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Obesity, Inflammation & Diabetes (1:30 PM-3:30 PM)

Title Vitamin D Deficiency Contributes to Insulin Resistance and NAFLD in Obese Rats

Author String CL Roth, C Elfers, SJ Melhorn, D Lattemann, A Hoofnagle, M Yeh, GJ Morton, J Nelson, K Kowdley
University of Washington, Seattle, WA; University of Washington, Seattle, WA; University of Washington, Seattle, WA; University of Washington, Seattle, WA; Virginia Mason Medical Center, Seattle, WA

Body Childhood obesity is a major health concern as it leads to long-term health consequences including the development of co-morbid diseases including non-alcoholic fatty liver disease (NAFLD), steatohepatitis (NASH) and type 2 diabetes mellitus (T2DM). While the mechanisms mediating these effects are not fully understood, recent clinical studies have demonstrated a high rate of vitamin D deficiency (VDD) among overweight children and that VDD contributes to the development of insulin resistance. To explore this further, we exposed young (starting at age 25d) Sprague-Dawley rats to either a low-fat diet (LFD) that was either replete (LFD) or deficient in vitamin D (LFD-VDD) for 10 weeks. To mimic the diet typically consumed by overweight/obese children, a second group of rats were exposed to high-fat diet (HFD, 45% fat) supplemented with high-fructose corn syrup (HFCS)-sweetened beverages (12.5%), that were either vitamin D deficient (HFD-HFCS-VDD) or not (HFD-HFCS) for 10 weeks (n=10/group). At the end of the treatment, we performed intraperitoneal insulin tolerance (ITT) and glucose tolerance (GTT) tests. Liver histology including steatosis, lobular inflammation, hepatocellular ballooning, and fibrosis was also assessed using the NASH CRN scoring system by a blinded hepatopathologist. As expected, total vitamin D levels were reduced in VDD groups to 13-47% compared to animals receiving normal dietary vitamin D. While LFD-VDD animals gained similar weight to LFD animals, they exhibited insulin resistance ($p < 0.05$). As expected HFD-HFCS animals gained more body weight and were characterized by insulin resistance, glucose intolerance and fatty liver relative to LFD animals ($p < 0.05$, for each). However, vitamin D deficient HFD-HFCS-VDD animals exhibited more lobular inflammation relative to HFD-HFCS rats. These data suggest that vitamin D deficiency can contribute to the development of insulin resistance and increases the risk of NAFLD/NASH, even independent of weight gain. Treating individuals with vitamin D may be a potentially effective therapeutic for reducing insulin resistance and/or NAFLD/NASH in obese individuals.

Nothing to Disclose: CLR, CE, SJM, DL, AH, MY, GJM, JN, KK

Pub # P3-393

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Obesity, Inflammation & Diabetes (1:30 PM-3:30 PM)

Title Reduction of Visceral Adipose Macrophage Content by Clodronate Liposomes Improves Metabolic Profile in Diet-Induced Obese Mice

Author String B Feng, P Jiao, Y Nie, Z Yang, H Xu
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Body Obesity-related insulin resistance and type 2 diabetes have become a major public health burden. Chronic adipose inflammation has been identified as a causal factor for the development of insulin resistance in obese state. Accumulation of macrophages in adipose tissue is the critical source of inflammation. To determine the effect of adipose tissue macrophage depletion on insulin sensitivity in diet-induced obese (DIO) mice, clodronate liposomes were used to ablate macrophages in visceral adipose tissue of lean and DIO mice. Macrophage contents in adipose tissue, metabolic parameters, glucose and insulin tolerance, chemokine and cytokine production were measured. Injection of clodronate liposomes was able to reduce macrophage content in visceral adipose tissue of DIO mice. Reduced macrophage content was associated with reduced blood glucose levels, mildly improved insulin sensitivity, decreased hepatic triglyceride content, decreased MCP-2 level and increased adiponectin level. Reduction of macrophage content in adipose tissue of DIO mice is beneficial for improving metabolism.

Nothing to Disclose: BF, PJ, YN, ZY, HX

Pub # P3-394

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Obesity, Inflammation & Diabetes (1:30 PM-3:30 PM)

Title Metabolic Benefits Can Be Attained by Exercise Training with No Dietary Changes in Rats with the Metabolic Syndrome

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Body The treatment of metabolic syndrome includes lifestyle changes (low-caloric diet and exercise training), but the individual benefits of these interventions are usually not studied in separate. Aim: To evaluate the effects of aerobic exercise training, without dietary changes, on insulin sensitivity, GLUT4 in insulin-sensitive tissue and cardiovascular variables in an animal model of metabolic syndrome. Methods: Twenty male spontaneously hypertensive rats (SHR) received monosodium glutamate (MS) or saline (H) during their neonatal period for 10 days. Six months later they were randomized to MS (MS maintained sedentary), MS-T (MS trained on a treadmill for 1 hour, 5 days/week, 10 weeks), H (H maintained sedentary) and H-T (H trained on a treadmill for 1 hour, 5 days/week, 10 weeks). Weight, Lee index, blood pressure (tail-cuff system), insulin sensitivity (insulin tolerance test) and functional capacity (maximal exercise test) were evaluated before and after the exercise training protocol. The rats were euthanized with a high pentobarbital dose and tissues (heart, white adipose tissue and gastrocnemius muscle) were removed for GLUT4 analyses (Western blot). Results: The monosodium glutamate-treated SHR showed metabolic syndrome characteristics central obesity, increased Lee index: 0.28 ± 0.01 , H vs. 0.33 ± 0.1 , MS ($p < 0.05$), arterial hypertension and insulin resistance (kITT: 3.9 ± 0.5 , H vs. 2.8 ± 0.4 , MS, $p < 0.05$). MS rats had low GLUT4 expression in all tissues examined, as compared with H rats ($p < 0.05$). Exercise training determined body weight (10%, $p < 0.05$) and Lee index (12%, $p < 0.001$) reductions in MS-T compared to MS, and also systolic blood pressure lowering (12% MS-T vs. MS; 13% H-T vs. H). In exercise-trained animals, insulin sensitivity increased (4.7 ± 0.3 , H-T vs 3.8 ± 0.5 , H, $p < 0.05$ and 4.3 ± 0.6 , MS-T vs 2.9 ± 0.9 , MS, $p < 0.05$), as well as was GLUT4 expression in the heart (67% MS-T vs. MS; 48% H-T vs. H), white adipose tissue (386% MS-T vs. MS; 86% H-T vs. H) and gastrocnemius muscle (237% MS-T vs. MS; 98% H-T vs. H). There was a negative correlation between Lee index and GLUT4, mainly in fat tissue ($r = 0.81$, $p < 0.001$) and a positive correlation between insulin sensitivity and GLUT4, especially in the gastrocnemius ($r = 0.76$, $p < 0.001$). Conclusions: Exercise training alone improved the metabolic and cardiovascular alterations seen in the experimental model of metabolic syndrome, which involves enhanced GLUT4 protein expression.

Nothing to Disclose: BS, PWC, AML, GHP, MMM, UFM

Pub # P3-395

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Obesity, Inflammation & Diabetes (1:30 PM-3:30 PM)

Title A Stable Analog of Adropin Does Not Improve Glucose Homeostasis in Diet-Induced Obese Mice

Author String F Tremblay, CX Ross, X Li, SE Will, D Li, A-M LaRonde-Richard, K Magure, M Perreault, RE Gimeno, JF Tobin, S Wang
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Body Adropin is a highly conserved secreted protein expressed in both liver and brain. Transgenic overexpression of adropin, as well as administration of a synthetic peptide, adropin₃₄₋₇₆, has been shown to improve glucose homeostasis in mice. To further understand the biological functions of adropin, we evaluated the effect of adropin₃₄₋₇₆ in primary human hepatocytes and mouse pancreatic islets. Treatment of primary human hepatocytes with adropin₃₄₋₇₆ had no effect on PEPCK expression, a key regulator of gluconeogenesis, and did not modulate the glucagon response. We then examined the effect of adropin on glucose-stimulated insulin secretion in islets and found that adropin₃₄₋₇₆ does not acutely modulate insulin secretion *ex vivo*. To clarify the therapeutic potential of adropin, we designed two adropin fusion protein, Fc-adropin (free C-terminus) and adropin-EK-Fc (free N-terminus). Both adropin Fc fusion proteins were found to be stable in mouse serum *in vitro* and *in vivo* (>24 hours). Short-term treatment with either fusion proteins (one injection, 5 mg/kg, *i.p.*) had no effect on glucose excursion during an oral glucose tolerance test (OGTT) in diet-induced obese mice. Interestingly, injection of adropin-EK-Fc but not Fc-adropin resulted in higher insulin levels during the OGTT suggesting that adropin might reduce insulin sensitivity (*i.e.* higher insulin requirement for similar glucose excursion). To further establish the role of adropin on glucose homeostasis, diet-induced obese mice were treated once daily with adropin-EK-Fc for four consecutive days. Short-term therapy with adropin-EK-Fc had no effect on body weight, as well as on glucose and insulin levels during the OGTT. Our data shows that short-term administration of adropin fusion proteins in obese mice does not improve glucose homeostasis or affect body weight. Identification of the receptor and/or mechanisms by which adropin acts will be required to further assess its potential as a therapeutic treatment for obesity and obesity-related metabolic disorders.

Nothing to Disclose: FT, CXR, XL, SEW, DL, A-ML-R, KM, MP, REG, JFT, SW

Pub # P3-396

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Obesity, Inflammation & Diabetes (1:30 PM-3:30 PM)

Title Chronic Central Leptin Infusion Differently Modulates Brain and Liver Insulin Signaling

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Body Recent studies reported the impact of leptin on peripheral insulin sensitivity and glucose utilization. However, little is known concerning the effect of central leptin on hypothalamic and hepatic insulin efficiency. This study aimed to determine the consequence of intra-cerebroventricular (ICV) chronic leptin infusion on hypothalamic and hepatic insulin signaling pathways.
The impact of a long term ICV leptin infusion on insulin signaling was investigated in vivo in rats. A two-week central leptin infusion enhanced insulin-dependent Akt phosphorylation in the liver without changing PTP-1B protein expression, associated to insulin receptor upregulation and reduced IRS-1 phosphorylation on Ser302 residue. In the hypothalamus, chronic ICV leptin infusion induced PTP-1B protein expression associated with a specific decrease in insulin-dependent Akt phosphorylation.
Our results underline a brain leptin-dependent increase in hepatic insulin efficiency as mirrored by IR up-regulation, increased insulin-dependent Akt phosphorylation and reduced IRS-1 phosphorylation on serine 302 residue.

Nothing to Disclose: FB, CR, AG, KG, MT

Pub # P3-397

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Obesity, Inflammation & Diabetes (1:30 PM-3:30 PM)

Title Long-Term Ketogenic Diet (22 Weeks) in Mice Leads to Weight Gain, Hepatic Steatosis and Impaired Glucose Tolerance

Author String BEPB Ballieux, L van Dijck, R den Doelder, E de Koning
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Body The ketogenic diet (KD) is an effective treatment for refractory epilepsy in children. The high fat and low carbohydrate diet leads to a permanent state of ketosis and patients switch from a carbohydrate-based energy metabolism to a fat-based energy metabolism. Ketones are thought to play a central role in the anticonvulsant effect of the KD. Side effects of the diet include inadequate growth, hypoglycemia, weight loss, muscle wasting and osteoporosis. Metabolic changes by long term KD treatment, such as an increase in visceral fat and features of metabolic syndrome, might trigger loss of efficacy in seizure reduction. However, these metabolic changes and the mechanism for the loss of efficacy have not been investigated in detail in humans or mice.

To evaluate the long term metabolic effects of prolonged KD feeding, male C57Bl6 mice were fed a KD (n=10) or regular chow (n=10) for 22 weeks. Liver fat was determined and metabolic parameters (ITT, GTT) were assessed. During the first 2 weeks, mice on KD had a profound decrease in weight (26.9 g to 21.7 g, $p<0.001$) compared to the increase in control mice (25.7 g to 27.5 g, $p<0.001$). Subsequently, KD-fed mice started to gain weight leading to a significant higher weight at 22 weeks compared to mice fed a chow diet (35.6 g vs. 32.3 g, $p<0.05$). At 22 weeks the ITT was not different between both groups. Basal glucose concentrations did not differ significantly between KD and chow (6.4 mmol/l vs. 7.0 mmol/l, $p=0.27$), but glucose tolerance was impaired in the KD-fed mice compared to the controls (AUC 3244 vs. 2049, $p<0.0001$). The insulin secretory response during the first half hour of the GTT was decreased in KD vs. chow (delta insulin 6.4 mIU/l vs. 11.5 mIU/l, $p<0.05$). The insulin secretory response was negatively correlated to the AUC of glucose in the GTT of KD-fed mice ($r=-0.81$, $p<0.02$). A twofold increase in liver triglycerides in KD-fed mice was observed (57.6 nmol/g vs. 29.0 nmol/g, $p<0.0001$). The liver triglyceride content was not correlated to either AUC of glucose or delta insulin in the GTT in KD-fed mice.

In conclusion, a long-term KD in mice increases body weight and hepatic steatosis but does not lead to insulin resistance assessed by ITT compared to control mice. The impaired GTT observed during a ketogenic diet could be due to an abnormal insulin secretory response.

Nothing to Disclose: BEPB, LvD, RdD, EdK

Pub # P3-398

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Obesity, Inflammation & Diabetes (1:30 PM-3:30 PM)

Title Investigating Estrogen's Protective Effect on Glucose Homeostasis in a Genetic Model of Hyperglycemia

Author String J Newton, JP Warne, AW Xu
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Body Amongst many critical functions in regulating energy balance, leptin receptor-expressing neurons in the brain are also responsible for helping to maintain whole-body glucose homeostasis. In order to better understand how central leptin signaling controls insulin release and sensitivity in the periphery, we performed studies using transgenic mutant mice lacking Stat3 in neurons expressing the long-form leptin receptor (LepRb-Stat3). Though mutants of both sexes are significantly obese, female mutants appear to be protected from the some of the expected comorbidities, including hyperglycemia and decreased longevity observed in male mutants. Male mutants display impaired glucose tolerance and insulin tolerance which worsens during adulthood. Interestingly, though females are able to maintain normal plasma glucose levels throughout life, there is a transient hyperglycemia which occurs during pregnancy. Taken together, the aforementioned data, as well as other experiments which will be presented, suggest that estrogen may act both directly on the pancreas and indirectly through leptin receptor neurons to regulate glucose homeostasis.

Sources of Research Support: Hurlbut-Johnson Foundation to A.W.X.; IRACDA fellowship NIGMS 5K12GM081266-02 to A.J.N.; core facilities funded by NIH DERC P30 DK063720.

Nothing to Disclose: JN, JPW, AWX

Pub # P3-399

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Obesity, Inflammation & Diabetes (1:30 PM-3:30 PM)

Title Oral, but Not Injectable, Salmon Calcitonin Strongly Improves Glucose Homeostasis and Insulin Sensitivity in Obese Diabetic Rats

Author String MJ Feigh, K Henriksen, KV Andreassen, C Hansen, JE Henriksen, H Beck-Nielsen, C Christiansen, MA Karsdal
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Body The purpose of these studies was to investigate acute and chronic anti-diabetic effects of a novel oral formulation of salmon Calcitonin (sCT) in diet-induced obese (DIO) and Zucker Diabetic Fatty (ZDF)-rats. We hypothesized that oral, but not injectable form of sCT, by following the physiological enteric pathway, would provide better pharmacodynamic efficacy. Initially, in lean healthy rats, acute treatment with oral sCT (0.5, 1, and 2 mg/kg sCT combined with the oral carrier 150 mg/kg 5-CNAC) and injectable sCT (5 [μ]g/kg) showed a diabetogenic effect by increasing plasma glucose levels compared to vehicle ($p < 0.01$). In contrast using DIO-rats, acute treatment with oral sCT, but not injectable sCT nor amylin (5 [μ]g/kg) markedly improved glucose tolerance by reducing glucose and insulin area under the curve (AUC) during oral glucose tolerance test (OGTT) with 65% and 85%, respectively, compared to vehicle ($p < 0.001$). Following 4 weeks of chronic treatment in DIO-rats, oral sCT, but not injectable sCT, significantly and dose dependently reduced fasting and non-fasting glucose and insulin levels with 1.5 mM and 65%, respectively, compared to vehicle ($p < 0.01$). Concomitantly oral sCT, but not injectable sCT, improved insulin sensitivity (HOMA-IR) and normalized pancreatic β -cell function (HOMA- β). Compared to vehicle, oral and injectable salmon calcitonin resulted in a 15% decrease in body weight in the 4 week treatment period. A chronic proof of concept study in seven-week old ZDF-rats showed that plasma insulin levels were reduced by app. 60% in vehicle treated animals, demonstrating severe pancreatic dysfunction. This was totally prevented by oral sCT treatment ($p < 0.01$). Furthermore, oral sCT reversed hyperglycemia by reducing fasting plasma glucose with 5.4 mmol/l (11.2 mmol/l vs 5.8 mmol/l) ($p < 0.01$) and by reducing HbA1C levels with 1.7% (7.48% vs 5.75%) compared to vehicle group. During OGTT, oral sCT dose-dependently improved glycemic control by reducing glucose AUC by 60% and increasing insulin AUC 50% compared to vehicle group ($p < 0.001$). The present findings introduce a novel oral form of salmon Calcitonin as a promising agent for the reversal of hyperglycemia and thus prevention of type 2 diabetes.

Sources of Research Support: The Danish Research Foundation.

Disclosures: MJF: Employee, Nordic Bioscience. KH: Employee, Nordic Bioscience A/S. KVA: Employee, Nordic Bioscience A/S. CH: Employee, Nordic Bioscience A/S. CC: Chairman, Nordic Bioscience A/S. MAK: Employee, Nordic Bioscience A/S; Employee, Nordic Bioscience A/S. Nothing to Disclose: JEH, HB-N

Pub # P3-400

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Obesity, Inflammation & Diabetes (1:30 PM-3:30 PM)

Title Effects of GABA_B Agonists and Antagonists on Glycemia Regulation in Mice

Author String MM Bonaventura, M Crivello, ML Ferreira, C Libertun, V Lux-Lantos
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Body g-Aminobutyric acid (GABA) has been proposed to inhibit insulin secretion through GABA_B receptors (GABA_BRs) in pancreatic b-cells. GABA_BR knock-out mice show disruptions in glucose homeostasis (1). In clinical practice GABA_B agonists are used in the treatment of anxiety, dream disorders and neuropathic pain; antagonists improve cognitive performance. Little is found in the literature analyzing the effect of GABA_B analogues on glucose homeostasis *in vivo*, so this was the aim of our studies. BALB/c mice were chronically or acutely injected with GABA_B agonist baclofen (Bac: 5 and 7.5 mg/kg of body weight -bw-) and/or antagonist 2-hydroxisaclofen (2OH: 10 and 15 mg/kg bw). Blood glucose was measured by a One touch[reg] Ultra[trade] glucose meter from tail blood, serum insulin and glucagon by ELISA. Pancreatic islets were isolated from mice and glucose-stimulated insulin secretion tests (GSIS) were evaluated by RIA, in presence or absence of GABA_B analogs.

Mice acutely treated with Bac presented glucose intolerance [glycemia (mg/dl) at 75 min: Bac, 187 ± 16 vs sal 122 ± 5, Two-way ANOVA for repeated measures (RM), p<0.05] and diminished insulin secretion (serum insulin: Bac vs sal, two way ANOVA-RM, interaction: ns, Treatment factor p<0.05) during a glucose tolerance test (GTT). 2OH improved GTTs (glycemia at 75 min: 2OH, 113 ± 5 vs sal 155 ± 10, Two-way ANOVA-RM, p<0.05) and reversed the effect of Bac (glycemia at 75 min: Bac, 200 ± 16 vs sal, 166 ± 11, p<0.05; 2OH-Bac, 176 ± 7 vs sal, 166 ± 11, ns. Two-way ANOVA-RM). Also a slight increase in insulin secretion was observed with 2OH. In chronically-treated animals both the agonist and the antagonist induced impaired GTTs (glycemia: Bac and 2OH vs sal, two way ANOVA-RM, interaction: ns, Treatment factor p<0.05); the antagonist, but not the agonist, also induced a decrease in insulin secretion (serum insulin: 2OH vs sal, two way ANOVA-RM, interaction: ns, Treatment factor p<0.05). No alteration in insulin tolerance tests, body weight or food intake was observed with treatments. Bac inhibited glucose-stimulated insulin secretion and 2OH reversed the effect of Bac, both without affecting basal levels.

Results demonstrate that GABA_BRs are involved in the regulation of glucose homeostasis *in vivo*. Treatment with agonists or antagonists, given acutely or chronically, altered glucose homeostasis and insulin secretion alerting to the need to evaluate glucose metabolism during clinical practice.

Nothing to Disclose: MMB, MC, MLF, CL, VL-L

Pub # P3-401

Session Information POSTER SESSION: BASIC/TRANSLATIONAL - Obesity, Inflammation & Diabetes (1:30 PM-3:30 PM)

Title Role of Cardiotrophin-1 in Regulating Insulin Sensitivity in Skeletal Muscle, Adipose Tissue and Liver

Author String M Bustos, MJ Moreno-Aliaga, N Perez-Echarri, B Gomez-Marcos, E Lopez-Franco, J Prieto
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Body Cardiotrophin-1 (CT-1) is a protein member of the interleukin (IL)-6 cytokine family and signals through the gp130/leukemia inhibitory factor receptor (LIFR) heterodimer. The formation of gp130/LIFR complex leads to the activation of Janus kinases/STAT, MAPK (ERK1/2) signaling pathways as well as signal transduction pathways important in energy balance such as PI3K and mTOR. Although all gp130 cytokines share the transmembrane protein gp130 as a transducer, the role of these cytokines in regulating insulin sensitivity is quite different and also controversial. Thus, ciliary neurotrophic factor (CNTF), a member of this family, has been reported to improve insulin resistance. However, other cytokines of the same family such as interleukin (IL)-6 or neuropoietin have been shown to induce insulin resistance. CT-1 has effects on adipocytes and we have shown that signals on the hepatocytes. In order to investigate if CT-1 could have a potential therapeutic role in insulin resistance we analyzed the glucose lowering properties of this cytokine in several models of hyperglycemia and the role of CT-1 in regulating insulin sensitivity after acute and chronic treatment on skeletal muscle, adipose tissue and liver. We found that acute CT-1 treatment prior insulin stimulation increased the insulin-induced P-AKT signaling in skeletal muscle, adipose tissue and liver. However, we observed that chronic administration of CT-1 did not modify the insulin-induced P-AKT and ERK1/2 signaling in adipose tissue. In vitro studies showed that CT-1 was able to increase insulin-induced glucose uptake in L6E9 myotubes in normal and insulin resistance conditions. rCT-1. In conclusion, CT-1 modulates the glucose uptake system in skeletal muscle, liver and adipose tissue. Because skeletal muscle accounts for the majority of glucose disposal in the body and, therefore it is the major site for suffering insulin resistance, these novel observations suggest that CT-1 may be consider as a potential therapy for insulin resistance.

Sources of Research Support: FIS Grant P1041321; Mutua Madrile[ntilde]a Grant; Gobierno de Navarra (Health Department); Linea Especial Nutricion, Obesidad y Salud.

Nothing to Disclose: MB, MJM-A, NP-E, BG-M, EL-F, JP

Pub # P3-402

Session Information POSTER SESSION: CLINICAL - Obesity: Causes & Consequences (1:30 PM-3:30 PM)

Title Relationships between IGF Axis Parameters, Inflammatory Markers, and Adipocytokines in Obese Adolescents

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Body Obese adolescents are at risk of cardiovascular disease (CVD). Our study aims to explore the links between insulin-like growth factor I (IGF-I), IGF binding protein 1 (IGFBP-1), and novel markers of CVD risk in this population. Low IGF-I is associated with impaired glucose tolerance and CVD, and low IGFBP-1 has been linked with metabolic syndrome. The IGF and inflammatory systems have close biological links, and pro-inflammatory markers like high sensitivity c-reactive protein (hsCRP) independently predict CVD and diabetes. Adipocytokines are also important markers. Adiponectin correlates inversely with insulin resistance (IR), and higher levels independently predict reduced risk of CVD. It also demonstrates anti-inflammatory and myocardial remodeling effects. Abnormal leptin signaling is implicated in obesity-related CVD. We hypothesize that IGF-I and IGFBP-1 are related to these proteins. Because the IGF family is linked to inflammation, we suspect that these proteins affect coronary vessels independent of insulin and body mass, and may serve as novel markers of CVD risk.

We recruited 63 obese adolescents age 8-17yrs; 44% were male. Race: 1 Asian, 35 Black, 23 White. Ethnicity: 8 Hispanic. Blood was drawn at 8am fasting. Means (standard deviations) were: weight 101.3kg (21.7), BMI 36.7kg/m² (6.7), IGF-I 316.4ng/mL (119.3), IGFBP-1 6.45ng/mL (11.3), hsCRP 5.32mg/L (5.18), adiponectin 4.5ug/mL (1.7), leptin 37.6ng/mL (17.9). Using Spearman bivariate correlations we saw strong correlation between IGF-I and hsCRP ($r=-0.470$, $p<0.0005$), and also between IGF-I and leptin ($r=-0.297$, $p=0.028$), but not between IGF-I and adiponectin ($r=0.006$, $p=0.96$). We also found significant correlation between IGFBP-1 and leptin ($r=-0.274$, $p=0.045$); however, following Spearman partial correlations controlling for BMI and fasting insulin the relationship was no longer significant. IGFBP-1 and adiponectin were strongly correlated ($r=0.523$, $p<0.0005$). Following Spearman partial correlations controlling for fasting insulin ($r=0.456$, $p=0.0008$) and for BMI z-score ($r=0.526$, $p<0.0005$) the relationship remained highly significant.

Our findings show a strong relationship between IGFBP-1 and adiponectin independent of BMI and insulin, and between IGF-I and hsCRP/leptin. This supports our hypothesis that the IGF axis is related to markers of cardiovascular risk. Higher IGF-I levels in adults may serve as a vascular protective factor; further study is needed in youth at risk for CVD.

Nothing to Disclose: PA, DK, PCB, PRG, SNM, LELK

Pub # P3-403

Session Information POSTER SESSION: CLINICAL - Obesity: Causes & Consequences (1:30 PM-3:30 PM)

Title Cell Death-Inducing DNA Fragmentation Factor Alpha-Like Effector A (CIDEA) V115F (G>T) Polymorphism Associated with Obesity and Metabolic Alterations in Brazilian Obese Children and Adolescents

Author String SM Diniz, S Deram, I Guazzelli, E Frazzatto, T Arthur, S Villares
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Body

Introduction: Cell death-inducing DFF45-like effector (CIDE) is involved in the regulation of lipid metabolism¹. CIDEA gene expression decreases in individuals with phenotypes of obesity and metabolic syndrome (MS)^{2,3}. Lately, Dahlman I. et al detected in Swedish subjects a single nucleotide polymorphism (SNP), CID19787G>T, which encodes an exon 4, valine 115 to phenylalanine (V115F) amino acid substitution⁴. It was found an involvement of G allele in human obesity. However, studying a group of Japanese men, allele T was associated to phenotypes of MS⁵. The aim of this study was to evaluate the influence of SNP of CIDEA V115F (G>T) gene upon anthropometric and metabolic parameters in Brazilian obese children and adolescents (OCA).

Methods: 281 OCA participated in a 20 weeks weight-loss program [10.7±1.4yrs, body mass index (BMI) = 30.5 ± 4.5 kg/m², BMI Z-score 2.32±0.28, 49% pubescent, 100 boys]. The V115F (G>T) has been evaluated by polymerase chain reaction (PCR) and sequencing PCR fragments has been carried out by Applied Biosystems 3130XL Genetic Analyzer Outcome measures included degree of overweight, abdominal obesity, quantity of body fat, dyslipidaemia, disturbed glucose metabolism, insulin sensitivity and resistance, adipocyte-secreted hormones and dietary ingestion. Parametric and Independent t test, and Multiple Linear Regression were performed with SPSS.

Results: Allele distribution was in Hardy-Weinberg equilibrium ($\chi^2=2.17$; p=0.14). ZBMI (GG:2.36±0.29; GT+TT:2.29±0.28; p=0.003), body fat percentage of bioelectrical impedance (GG:37.7±4.10; GT+TT:36.3±4.23; p=0.005) and high-density lipoprotein (HDL)-C (mg/dl) (GG:39.9±9.18; GT+TT:42.7±9.21; p=0.01) showed significant differences between the haplotypes in OCA. However, after the adjustment of the variables by age, pubertal status and sex, CIDEA V115F (G>T) polymorphism was correlated with BMI (kg/m²) (b=-0.12; p=0.03), ZBMI (b=-0.12; p=0.05) and HDL-C (mg/dl) (b=0.15; p=0.01). After 20 weeks of weight-loss program in OCA, an improvement of metabolic and anthropometric parameters was observed. Even though the haplotype GG showed a smaller reduction of waist circumference (cm) than GT+TT (GG:2.21±4.45,GT+TT:4.39±5.81;p=0.05).

Conclusions: The haplotype GG of CIDEA V115F polymorphism gene was associated with a higher degree of obesity and this effect was manifested with a more severe metabolic profile. Our findings suggest a complex genotype - environmental interactions on obesity risk.

1 Wolins NE, Brasaemle DL, Bickel PE 2006 A proposed model of fat packaging by exchangeable lipid droplet proteins. FEBS Letters 580: 5484-5491. 2 Gummesson A, Jernas M, Svensson PA, Larsson I, Glad CAM, Chele E, Gripeteg L, Sjöholm K, Lystig TC, Sjöström L, Carlsson B, Fagerberg B, Carlsson LM 2007 Relations of adipose tissue CIDEA gene expression to basal metabolic rate, energy restriction and obesity: population-based and dietary intervention studies. The Journal of Clinic Endocrinology and Metabolism 92: 4759-4665. 3Nordstrom EA, Ryden M, Backlund EC, Dahlman I, Kaaman M, Blomqvist L, Cannon B, Nedegaard J, Arner P 2005 A human-specific role of cell death inducing DFFA (DNA fragmentation factor-alpha)-like effector A (CIDEA) in adipocyte lipolysis and obesity. Diabetes 54: 1726-1734. 4Dahlman I, Kaaman M, Jiao H, Kere J, Laakso M, Arner P 2005 The CIDEA gene V115F polymorphism is associated with obesity in Swedish subjects. Diabetes 54: 3032-3034. 5Zhang L, Miyaki K, Nakayama T, Muramatsu M 2008 Cell death-inducing DNA fragmentation factor α -like effector A (CIDEA) gene V115F (G[rarr]T) polymorphism is associated with phenotypes of metabolic syndrome in Japanese men. Metabolism Clinical and Experimental 57: 502-505.

Nothing to Disclose: SMD, SD, IG, EF, TA, SV

Pub # P3-404

Session Information POSTER SESSION: CLINICAL - Obesity: Causes & Consequences (1:30 PM-3:30 PM)

Title Metabolic and Genetic Predictors of Circulating Adipocyte Fatty Acid-Binding Protein

Author String A Tonjes, S Kralisch, U Lossner, P Kovacs, M Bluher, M Stumvoll, M Fasshauer
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Body Adipocyte fatty acid-binding protein (AFABP) was recently introduced as a novel adipokine playing an important role in glucose homeostasis. In the current study, we investigated the relationship between serum AFABP levels and metabolic, as well as cardiovascular parameters, in the self-contained population of Sorbs. Furthermore, we analyzed the effects of common variants in the FABP4 gene on AFABP serum concentration. Serum AFABP concentrations were quantified by ELISA and correlated with metabolic and cardiovascular parameters, as well as inflammatory markers and renal function in 868 well-characterized non-diabetic Sorbs from Germany. Median AFABP serum concentrations were 1.5-fold higher in female subjects (23.03 [micro]g/l) as compared to males (15.86 [micro]g/l). Waist-to-height-ratio and glomerular filtration rate were independently associated with AFABP concentrations in multiple regression analysis in both females and males. In females, the number of children born was a positive predictor of circulating AFABP independent of body fat mass. Two single nucleotide polymorphisms, rs16909187 and rs10808846 representing common genetic variation in FABP4 did not show any effects on serum AFABP concentrations in our study cohort. Taken together, AFABP serum concentrations are determined by parameters of fat distribution, renal function, gender, and potentially number of pregnancies.

Nothing to Disclose: AT, SK, UL, PK, MB, MS, MF

Pub # P3-405

Session Information POSTER SESSION: CLINICAL - Obesity: Causes & Consequences (1:30 PM-3:30 PM)

Title Serum Galectin-3 in PWS Is Positively Correlated with BMI and Percent Body Fat

Author String D Kim, SH Kim, SY Cho, S Ji, YB Sohn, K-H Paik, D-K Jin
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Body Prader-Willi syndrome (PWS) is the most common genetic cause of obesity in humans. Patients with the PWS have abnormal body composition with high percentage of body fat. Galectin-3 has recently been identified to be secreted from adipocytes and is elevated with obesity. The aim of this study was to assess circulating galectin-3 levels in patients with PWS. Forty seven patients with PWS and twelve obese controls were enrolled. Growth was analyzed and galectin-3 was measured by ELISA. The percent body fat and laboratory studies including total cholesterol, glucose, and insulin, aspartate aminotransferase (AST), alanine aminotransferase (ALT) or IGF-1 were determined. There were no significant differences in the galectin-3 levels between the patients with PWS and controls. The patients with PWS had circulating galectin-3 levels that were positively correlated with the BMI ($P=0.007$, $r=0.384$) and the percentage of body fat ($P=0.003$, $r=0.648$). The results of this study showed that although galectin-3 plays an important role in the pathophysiology of obesity and may have the capacity to alter adipocyte cell numbers, systemic galectin-3 in PWS is not different from normal obese controls and was positively correlated with the BMI and percent body fat as well.

Nothing to Disclose: DK, SHK, SYC, SJ, YBS, K-HP, D-KJ

Pub # P3-406

Session Information POSTER SESSION: CLINICAL - Obesity: Causes & Consequences (1:30 PM-3:30 PM)

Title Racial Differences in Association between Deleterious Health Behaviors and Intra-Peritoneal Fat

Author String R Kazlauskaite, K Karavolos, I Janssen, KJ Shipp, S Dugan, LH Powell
Rush University Medical Center, Chicago, IL

Body The aim of this study was to explore the association between deleterious health behaviors (high energy intake, low physical activity and smoking) and intra-peritoneal adipose tissue area (IPAT), and the differential effect of race in this relationship.

We performed a cross-sectional analysis of 257 women, recruited between August 2002 and December 2005 for the SWAN Fat Patterning Study, a Chicago site ancillary substudy of the Study of Women's Health Across the Nation (SWAN). IPAT area was assessed by computed tomography; energy intake was assessed using the Block Food Frequency Questionnaire, physical activity was assessed using the Kaiser Physical Activity Survey, smoking assessed was by self-report. Linear regression models were used for the principal analyses. Among 257 women, 48 % (n=134) were African-American and 52 % (n=123) Caucasian, 53% had annual household income above \$75,000, and 61% had a college or higher degree. At the time of IPAT assessments women were an average of 52 years old, 46% were obese and 30% were overweight, 49% (n= 125) were post menopausal, and the remaining were pre- or peri-menopausal. Deleterious health behaviors were associated with higher IPAT: higher energy intake (every 500 calories of energy intake were associated with 6% higher IPAT, p=0.02), lower physical activity (each lower point on the KPAS score was associated with 4% higher IPAT, p=0.009) and cigarette smoking (12% higher IPAT in smokers, p=0.08), after adjustment for total body adiposity, age, income, race and menopausal status. There was a significant energy intake and race interaction (p=0.02). In stratified analysis, high energy intake was independently associated with higher IPAT in Caucasian, but not African American women, after adjustment for total body adiposity, age, income and menopausal status. Lower physical activity and smoking were also associated with IPAT in Caucasian, but no African American women.

In summary, our findings suggest that self-reported deleterious behaviors are associated with IPAT in Caucasian, but not in African American midlife women, independent of total body adiposity and sociodemographic factors. It appears that less is known about correlates of IPAT in African American than in Caucasian midlife women. Future studies should preferentially target African American women to understand lifestyle behavior correlates of IPAT.

Nothing to Disclose: RK, KK, IJ, KJS, SD, LHP

Pub # P3-407

Session Information POSTER SESSION: CLINICAL - Obesity: Causes & Consequences (1:30 PM-3:30 PM)

Title Mortality Risk Association between Exercise Capacity and Obstructive Sleep Apnea in Men with Type 2 Diabetes Mellitus and/or Hypertension

Author String KA Alswat, SG Khosla, ES Nysten, R Khosla, MS Balish, PF Kokkinos
Veterans Affairs Medical Center and George Washington University, Washington, DC; Veterans Affairs Medical Center, Washington, DC; Veterans Affairs Medical Center, Washington, DC; Veterans Affairs Medical Center, Washington, DC; Veterans Affairs Medical Center and Georgetown University, Washington, DC

Body

Background:
Obstructive sleep apnea (OSA), type 2 diabetes mellitus (T2DM) and hypertension (HTN) often co-occur. Recent findings suggest that patients with OSA have increased risk for all-cause mortality compared to individuals without OSA. Exercise capacity is inversely related to all-cause mortality in patients with T2DM and other co-morbidities. Information on the impact of fitness and mortality risk in patients with OSA and T2DM and/or HTN is scarce. Thus, we sought to assess the relationship between exercise capacity or physical fitness and the risk of mortality in the pts with OSA and T2DM and/or HTN.

Methods:
We identified 567 male veterans (mean age: 62±11 years) with documented OSA, DM and/or HTN who completed a normal graded exercise tolerance test (ETT) between 1996 and 2010 at Veterans Affairs Medical Center, Washington, DC. The cohort consisted of 445 African Americans and 122 white. We categorized them based on the peak metabolic equivalents (METs) achieved during ETT. Individuals who achieved [≥]5 METs were classified as Low-Fit (n=138; age=67±1), those who achieved 5.1-10 METs were classified as Moderate-Fit (n=321; age=61±10) and those with a peak exercise capacity of >10 METs as High-Fit (n=108; age=57±9).

Results:
Significant differences among the fitness categories were noted in age and smoking status. There were no differences in BMI, resting systolic and diastolic blood pressure, co-morbidities or medications. During the follow-up period of ~14 years (mean: 5.4±3.9) there were a total of 331 deaths (average mortality rate of 107 deaths/1000 person-years). The mortality rate was 75.4% in the Low-Fit, 55.5% in the Moderate-Fit and 45.1% in the High-Fit category. Fully adjusted Cox proportional hazard analysis revealed an inverse and graded association between exercise capacity (METs) and mortality. More specifically, the mortality risk was 13% lower for each 1 MET increase in exercise capacity (hazard ratio: 0.87; CI: 0.82-0.93). Furthermore, individuals in the Low-Fit category had 80% higher risk of death (hazard ratio=1.8; CI=1.2-2.5) when compared to those in the High-Fit category.

Conclusion:
In individuals with OSA, T2DM and/or HTN, low exercise capacity significantly increases mortality rate by as much as 80%. Conversely, for each one MET increase in exercise capacity mortality rate is lowered by 13%.

Nothing to Disclose: KAA, SGK, ESN, RK, MSB, PFK

Pub # P3-408

Session Information POSTER SESSION: CLINICAL - Obesity: Causes & Consequences (1:30 PM-3:30 PM)

Title Health-Related Physical Fitness in Middle-Aged Adult Males with Metabolic Syndrome

Author String LGG Porto, KSL Mileski, JLAESP Leitao, A Lofrano-Porto
Brazilian Supreme Labor Court, Brasília, Brazil; Brasília University - UnB, Brasília, Brazil; University Hospital of Brasília (HuB;UnB), Brasília, Brazil; Faculty of Health Sciences, University of Brasília - UnB, Brasília, Brazil

Body Metabolic syndrome (MS) is an increasingly common condition worldwide and is strongly related to obesity and a sedentary lifestyle. Health-related physical fitness (HRPF) components have been used as markers of physical health and their impairment is associated with increased morbi-mortality. **OBJECTIVE:** To compare HRPF levels between adult males with and without MS. **METHODS:** 79 men, 46.2±8.4 years, BMI: 26.7±3.9 kg/m², participants on a physical activity program of the Supreme Labor Court of Brazil ([ldquo]TST on The Move[rdquo]) were recruited for a cross-sectional evaluation. Volunteers were recruited among employees from security and transportation sections who have undergone annual physical activity evaluation. The five well-established HRPF components were evaluated by means of the VO₂max Ebbeling test (VO₂max) for cardiorespiratory capacity, sit-and-reach test for flexibility (SRT), handgrip dynamometer test (HDT) for muscular strength, push-up test (PUT) for muscular endurance and BMI for the body composition component. MS was defined as proposed by the IDF and AHA/NHLBI Joint Interim Statement (Circulation, 2009¹). As HRPF values were normally distributed (Shapiro-Wilk test), t-test was used. Odds Ratio (95% CI) (OR) was calculated in order to analyze the strength of the association between the presence of MS and the odds for being unfit (out of HRPF recommended values for sex and age). **RESULTS:** 19 volunteers with MS were identified (24.1%). VO₂max in the MS group was lower (38.6±5.4 ml/O₂/kg⁻¹) than that of the group without MS (44.2±5.2 ml/O₂/kg⁻¹; p<0,001), as was the PUT (14.4±5.6 vs 21.8±8.6 repetitions; p=0.001). Individuals without MS had lower BMI (25.9±3.6) than the MS-group (29.2±3.8 kg/m²; p=0.001). There were no differences in the HDT (52.2±6.7 vs 51.9±7.2 kg/f; p=0.9) nor in the SRT (24.5±5.9 vs 25.5±8.5 cm; p=0,6). The OR of being unfit according to VO₂max and BMI criteria in the MS group was 6.5 (1.9 - 22.6) and 5.7 (1.2 - 26.8), respectively. No significant differences were observed in flexibility and muscular strength between the groups: OR=1.7 (0.6 - 4.8) and OR=3.2 (0.9 - 12.1), respectively. **CONCLUSION:** Our data showed that 3 of the 5 HRPF components were lower in the MS group than in the group without MS. Moreover, these reduced values were associated with elevated odds of being out of the recommended values for VO₂max and BMI. Our data suggest an association between MS and important health related physical fitness components.

(1) Alberti KGMM et al., Circulation 2009; 1640-1645

Nothing to Disclose: LGGP, KSLM, JAESPL, AL-P

Pub # P3-409

Session Information POSTER SESSION: CLINICAL - Obesity: Causes & Consequences (1:30 PM-3:30 PM)

Title Metabolic Syndrome Is Inversely Related to Cardiorespiratory Fitness in US Career Firefighters

Author String DM Baur, SN Kales
Harvard School of Public Health, Boston, MA; The Cambridge Health Alliance, Cambridge, MA

Body

Background:
Cardiovascular disease (CVD) events account for 45% of on-duty fatalities among firefighters [1]. It has been demonstrated repeatedly that these events occur primarily in firefighters with underlying disease or excess cardiovascular risk factors [2]. Several studies have found a high prevalence of overweight plus obesity in the fire service, ranging from 73%-83%, as well as CVD risk factor clustering in obese firefighters [3,4].

Objective:
To assess the prevalence of Metabolic Syndrome (MetSyn) in career firefighters and its association with cardiorespiratory fitness (CRF).

Methods:
Cross-sectional cohort study of 965 Midwestern male career firefighters. CRF was measured objectively by maximal exercise tolerance testing and described as Standard Metabolic Equivalent (METS). CVD risk parameters included body composition and metabolic profiles (blood samples taken in fasting state). Group differences were compared using Chi-square test and logistic regression. Definition for MetSyn according to modified criteria from the American Heart Association [5] (where BMI [ge]30 was used instead of waist circumference [ge]102cm).

Results:
The average age of our cohort was 39.5 (SD 8.6), with an average BMI of 29.3 (SD 4.3). The prevalence of MetSyn was 28.1 %, while 21.8 % had none of the 5 criteria defining MetSyn. Firefighters in higher age groups had increased prevalence of MetSyn compared to younger colleagues (p-value 0.0005). Participants with MetSyn had an average METS of 11.1 (SD 1.8) compared to participants with no criteria [METS 12.9 (SD 1.7, p<0.0001 (adjusted for age)]. In the highest fitness category (METS>14) MetSyn was prevalent in only 5.2% compared to a nearly ten-fold higher prevalence, 51.2%, in the lowest fitness category (METS[le]10) [p-value <0.0001, adjusted for age].

Conclusion:
Our nation depends on the fire service to respond to major emergencies. We found a high prevalence of the MetSyn in this occupational group which is supposed to be fit and younger in age. There is a highly significant inverse association with CRF. As in the general population, there should be a strong incentive to enhance exercise and improve fitness. This would decrease the prevalence of MetSyn and most likely also decrease the future risk of on-duty CVD events.

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Sources of Research Support: Federal Emergency Management Agency (FEMA) Assistance to Firefighters Grant (AFG) program. The studies described in this report were conducted with support from awards EMW-2006-FP-01493 (PI: Dr. S.N. Kales), EMW-2009-FP-00835 (PI: Dr. S.N. Kales).

Nothing to Disclose: DMB, SNK

Pub # P3-410

Session Information POSTER SESSION: CLINICAL - Obesity: Causes & Consequences (1:30 PM-3:30 PM)

Title C-Reactive Protein vs. Other Obesity-Related Biomarkers in the Prediction of Cardiovascular Risk: A 6-Year Prospective Study in Hong Kong Chinese

Author String W-S Chow, MAM Yuen, AWK Tso, BMY Cheung, LSC Law, S-V Lo, KSL Lam
University of Hong Kong, Hong Kong, China; Queen Mary Hospital, Hong Kong, China; Hospital Authority, Hong Kong, China

Body

Introduction: Subclinical chronic inflammation, increasingly recognized as a key player in atherosclerosis, is found in obesity. C-reactive protein, measured using high-sensitivity assay (hsCRP), is the most promising inflammatory marker in predicting the risk of cardiovascular diseases (CVD). In this prospective cohort study we examined the predictive value of hsCRP for CVD in Hong Kong Chinese and determined if other obesity-related biomarkers would enhance the predictive value of hsCRP.

Research Design and Methods: Subjects were recruited from the Hong Kong Cardiovascular Risk Factors Prevalence Study 2 (CRISPS 2) cohort. Those with known cardiovascular disease(s) were excluded. Baseline serum levels of adiponectin, leptin, soluble tumour necrosis factor alpha receptor 2 (sTNFR2) and hsCRP were determined and subjects were followed prospectively for 6 years.

Results: 1785 subjects were included in the final analysis. The cumulative incidence of CVD was 3.4%. At baseline, subjects with incident CVD were older, and had higher body mass index (BMI), waist circumference (WC), systolic blood pressure (BP), HOMA-IR, and fasting glucose levels (all $p < 0.001$), compared to those who did not develop CVD (non-CVD). They also had higher baseline levels of leptin and sTNFR2 (both $p < 0.001$) and hsCRP (1.76 [0.87-2.55] mg/l vs. 0.69 [0.32-1.49] mg/l in non-CVD; median [interquartile range]; $p < 0.001$), but similar adiponectin levels, compared to non-CVD subjects. Logistic regression showed that baseline hsCRP was an independent predictor of CVD ($p = 0.003$) after controlling for the conventional CVD risk factors, and for leptin and sTNFR2. The predictive value of leptin or sTNFR2 was not significant after adjustment for conventional CVD risk factors, hsCRP and each other. Serum hsCRP levels tertile analysis (< 0.45 mg/l, 0.45 - 1.2 mg/l and > 1.2 mg/l) showed that, compared to subjects in the lowest tertile, those in the highest tertile had an odds ratio of 3.362 for incident CVD (95% CI=1.249-9.052; $p = 0.016$). Receiver operating characteristics (ROC) curve analysis found that an hsCRP level [ge] of 1 mg/l had the most optimal sensitivity and specificity for CVD prediction.

Conclusion: In this 6-year prospective study, hsCRP was an independent predictive factor of CVD in Hong Kong Chinese, in addition to conventional CVD risk factors. Measurement of adiponectin and other obesity-related biomarkers tested in this study did not provide any adjunctive predictive value.

Sources of Research Support: Health & Health Services Research Fund (#06070951).

Nothing to Disclose: W-SC, MAMY, AWKT, BMYC, LSCL, S-VL, KSL

Pub # P3-411

Session Information POSTER SESSION: CLINICAL - Obesity: Causes & Consequences (1:30 PM-3:30 PM)

Title Adipokines and Body Composition in South Asians: The Metabolic Syndrome and Atherosclerosis in South Asians Living in America (MASALA) Study

Author String AD Shah, D Mathur, MJ Budoff, AM Kanaya
Veteran Affairs Medical Center, San Francisco, CA; University of California, San Francisco, San Francisco, CA; Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, CA

Body

Introduction: Despite normal or low body mass index (BMI), South Asians have a higher amount of total body fat and abdominal fat compared to Caucasians. This may be associated with abnormal metabolic profiles and may be mediated by adipose secreted hormones known as adipokines, but has been poorly studied.

Methods: We performed a cross-sectional study of 150 South Asians, between the ages of 45 and 79 years, excluding those with pre-existing cardiovascular disease. Blood samples were obtained to measure glucose metabolism variables, lipid profiles, and adipokines. Total body fat was determined by dual-energy X-ray absorptiometry. Abdominal computed tomography was used to measure visceral fat area and hepatic fat (liver spleen ratio). We assessed the association between tertiles of adipokines and each body adiposity measure adjusting for age, sex, smoking history, hypertension, high-density lipoprotein (HDL), triglycerides, and homeostatic model assessment of insulin resistance (HOMA-IR).

Results: The average BMI was $26.1 \pm 4.6 \text{ kg/m}^2$ and did not differ by sex. Women had higher total body fat (26.4 ± 8.3 vs. $22.7 \pm 7.5 \text{ kg}$) while men had higher visceral fat area (155 ± 56 vs. $111 \pm 48 \text{ cm}^2$) and more hepatic fat (1.2 ± 0.2 vs. 1.3 ± 0.3 liver: spleen ratio). Women had higher leptin (21.6 ± 11.6 vs. $9.1 \pm 6.3 \text{ ng/ml}$) and adiponectin (9.0 ± 3.8 vs. $5.7 \pm 3.3 \text{ [micro]g/ml}$) than men. Higher tertiles of leptin were associated with higher BMI ($p < 0.001$) and higher total body fat ($p < 0.001$) and inversely associated with smoking ($p = 0.03$) and hypertension ($p = 0.01$). Higher tertiles of adiponectin were associated with lower HOMA-IR ($p < 0.01$), lower triglycerides ($p < 0.001$), higher HDL ($p < 0.001$), and lower adiposity (BMI ($p < 0.001$), total body fat ($p < 0.001$), visceral fat area ($p < 0.001$), and hepatic fat ($p = 0.01$)). After adjustment for covariates, leptin was significantly associated with a higher BMI (β 3.6 per SD; 95% CI 2.9-4.2), higher total body fat (β 6.4 per SD; 95% CI 5.4-7.3), higher visceral fat area (β 22.9 per SD; 95% CI 15.0-30.8) and more hepatic fat (β -0.05 per SD; 95% CI -0.1 - -0.01). After adjustment for HDL and triglycerides, adiponectin was no longer associated with any adiposity measure.

Conclusions: In a South Asian cohort, total and regional body fat were notably elevated despite only modestly elevated BMI. Leptin was independently associated with measures of adiposity while the inverse association of adiponectin with adiposity was significantly attenuated by HDL and triglycerides.

Nothing to Disclose: ADS, DM, MJB, AMK

Pub # P3-412

Session Information POSTER SESSION: CLINICAL - Obesity: Causes & Consequences (1:30 PM-3:30 PM)

Title Prolactin Levels Are Decreased in Patients with the Metabolic Syndrome

Author String LRM Oliveira, CF Mello, LB Fernandes, HR Celem, VT Oliveira, LCL Ciuffo Filho, TR Ferreira, ECO Naliato
UNIFESO (Serra dos [Oacute]rgãos University Center), Teresópolis, Brazil

Body An association between low serotonergic activity in the central nervous system (CNS) and the Metabolic Syndrome (MS) has been reported. Since serotonergic neurotransmission causes a release of pituitary hormones into circulation, prolactin (PRL) response during challenge tests can be used to estimate CNS serotonergic activity.
Objectives: To evaluate basal PRL levels in patients with and without the MS.
Patients & Methods: This cross-sectional study analyzed PRL, glucose, uric acid, total cholesterol, LDL, HDL, triglycerides, insulin, HOMA, systolic and diastolic blood pressure and anthropometric measures (abdominal circumference, body mass index - BMI) of 195 women (age = 43.1 ± 13.2 years, BMI = 29.4 ± 5.0 kg/m²) and 90 men (age = 38.1 ± 12.1 years, BMI = 31.9 ± 7.0 kg/m²) who sought Endocrine evaluation due to weight gain between January/2005 and October/2010. Based on the International Diabetes Federation (IDF) criteria, 31.3% of the women and 45.6% of the men were diagnosed with the MS. Biochemical and clinical data of patients with and without the MS were compared. Data were also analyzed simultaneously in order to identify correlations.
Results: Patients with the MS met 3.7 ± 0.8 IDF diagnostic criteria, while those without the MS met only 1.3 ± 0.8 ($p < 0.01$). Age and BMI were higher in patients with the MS than in those without the syndrome (age: 47.0 ± 13.6 vs 38.5 ± 11.8 years, $p < 0.01$; BMI: 33.5 ± 5.6 vs 28.4 ± 5.6 kg/m², $p < 0.01$). Patients with the MS had lower PRL levels than those without the MS (8.9 ± 4.8 vs 12.0 ± 5.7 ng/mL, $p < 0.01$). PRL levels were inversely correlated with those of glucose ($r = -0.27$), total cholesterol ($r = -0.16$), LDL ($r = -0.13$), triglycerides ($r = -0.16$), and HOMA ($r = -0.13$) and positively correlated with those of HDL ($r = 0.13$). PRL levels were also inversely correlated with BMI ($r = -0.20$), abdominal circumference ($r = -0.21$) and the number of IDF diagnostic criteria met by the patients ($r = -0.27$). In the multivariate analysis, the associations between PRL levels and the presence of the MS, glucose levels, and the number of IDF diagnostic criteria met by the patients persisted after the adjustment for the influence of BMI and age ($p < 0.01$).
Conclusion: Similarly to what has been observed during challenge tests, basal PRL is decreased in patients with the MS and may reflect the presence of a low CNS serotonergic activity in this syndrome.

Nothing to Disclose: LRMO, CFM, LBF, HRC, VTO, LCLCF, TRF, ECON

Pub # P3-413

Session Information POSTER SESSION: CLINICAL - Obesity: Causes & Consequences (1:30 PM-3:30 PM)

Title Assessment of the Relation between Serum Ghrelin Levels of Patients with Hashimoto Thyroiditis and Obesity

Author String HH Biyikli, S Isik, G Erden, U Ozuguz, F Dogru, A Arduc, D Berker, S Guler
Ministry of Health, Ankara Numune Research and Training Hospital, Ankara, Turkey; Ministry of Health, Ankara Numune Research and Training Hospital, Ankara, Turkey; Ministry of Health, Ankara Numune Research and Training Hospital, Ankara, Turkey

Body **Objective:** Hashimoto's thyroiditis (HT) is the most frequent cause of hypothyroidism and goiter in developed societies. Hashimoto's thyroiditis affects metabolic parameters and predisposes to obesity. In this study we aimed to assess the possible role of serum ghrelin (GHR) level in obesity in patients with HT.
Methods: Forty-eight patients with HT and age and sex matched 41 healthy individuals were included study. Anthropometric measurements of patients, their medical history, smoking status and medications were recorded. Cross-sectional laboratory variables including serum GHR, FT3, FT4, TSH, antiTg, antiTPO, fasting blood glucose (FBG) and insulin levels, and lipid parameters were assessed in all patients.
Results: Gender distribution, mean age, and BMI values of patients with HT and the control group were similar (women/men 42/6 vs. 33/8; 46.8±14.7 vs. 45 ± 12.5 years; 28.5 ± 6.1 vs. 28.4± 4.9 kg/m²), respectively; p>0.05 for all). Mean waist circumference (WC) values of patients with HT were significantly higher compared with the control group (100.6 ± 14.6 vs. 93.2±13.2 cm, p= 0.015). While FBG, LDL-C and TG levels in the group with HT were significantly higher compared with the control group, HDL-C and insulin levels and HOMA-IR values were similar. Mean TSH, Anti-TPO and Anti-TG levels were higher and mean FT4 level was lower in HT patients the controls, while mean FT3 level were similar. Serum GHR levels were lower in patients with HT compared with the control group (416.9 ± 224.4 and 689.9 ± 191.6 pg/ml, respectively; p<0.001). A negative correlation was observed between GHR level and BMI, WC and Anti-TPO levels. Free T4 levels demonstrated a positive correlation with GHR levels.
Conclusion: Our data show that HT patients have greater WC and lower GHR levels despite similar age and BMI; however, there is no correlation between WC and GHR levels. These data may indicate that altered body fat distribution in HT patients does not have a direct relation with GHR levels.

Nothing to Disclose: HHB, SI, GE, UO, FD, AA, DB, SG

Pub # P3-414
Session Information POSTER SESSION: CLINICAL - Obesity: Causes & Consequences (1:30 PM-3:30 PM)
Title Free T4 Levels Correlate with Fat Mass in Obese, Euthyroid Women
Author String R Anand, R Freeman, HR Mogul
Westchester Medical Center, Valhalla, NY; Montefiore Medical Center, Bronx, NY
Body **BACKGROUND** The relationship between weight and thyroid dysfunction is well established. However, few studies have assessed this association in the euthyroid state. **METHODS** We evaluated body composition using dual energy x-ray absorptiometry (DXA) and thyroid function in 51 women in the EMPOWIR (Enhance the Metabolic Profile of Women with Insulin Resistance) trial (NCT00618071). These women manifested insulin resistance at an early age - Syndrome W, defined by the triad of Weight gain (>20 lbs after the 20's), Waist gain, and White Coat hypertension; normal glucose tolerance tests (GTT's); and increased insulin response curves.¹ Study subjects meeting inclusion criteria - age 35-55; 20 lb weight gain; increased AUC-insulin; normal GTT were enrolled from Feb, 2008-Dec, 2009 in this 1-year, double blind, placebo controlled randomized clinical trial. Baseline characteristics of study participants at 2 sites were compared with analysis of variance (ANOVA). Multivariate models were used to compare study results for covariate adjustment of age, race, and initial BMI group (SPSS 13.0). **RESULTS** Total and free T4 correlated significantly with total fat, gynoid fat mass and %fat - legs and trunk, respectively $r_{xy} = -.295, -.313, -.297$, P 's = .040, .029, .038 and $r_{xy} = -.359, -.375, -.391$, P 's = .011, .008, .005). In contrast to other studies, we found TSH and T3 levels did not correlate with any component of body composition.²⁻⁴ **CONCLUSIONS** This data suggests that fat mass correlates significantly with thyroid function. This has important clinical implications raising the question of whether to keep the fT4 levels in the high normal range in symptomatic, obese females that continue to gain weight.

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Disclosures: HRM: Principal Investigator, GlaxoSmithKline; Study Investigator, Lilly USA, LLC; Consultant Pfizer, Inc. Nothing to Disclose: RA, RF

Pub # P3-415

Session Information POSTER SESSION: CLINICAL - Obesity: Causes & Consequences (1:30 PM-3:30 PM)

Title Metabolic Differences between Obese Patients with and without Obstructive Sleep Apnea Syndrome

Author String GB Aranda Velazquez, A Jimenez, V Perea Castilla, M Mora Porta, L Flores Meneses, J Ferrer Marrades, J Vidal
Hospital Clinic, Barcelona, Spain

Body **Background:** Whether the association between obstructive sleep apnea syndrome (OSAS) and the metabolic syndrome (MS) is simply the result of underlying obesity, or OSAS represents an additional burden that exacerbates metabolic dysfunction in severely obese subjects is not completely clear. **Objective:** The purpose of the present study was to evaluate the metabolic differences between severely obese subjects without OSAS or with newly diagnosed OSAS (not treated with CPAP), and between subjects with OSAS not treated or treated with CPAP. **Subjects and Methods:** 144 severely obese subjects were included in the present study. 34 individuals had been previously diagnosed of OSAS, and were treated with CPAP (t-OSAS). The remaining 110 were subjected to polysomnography, with 30 not fulfilling OSAS criteria (non-OSAS), and 80 being newly diagnosed with OSAS (nt-OSAS). The nt-OSA group were further classified according to the apnea-hipopnea index (AHI) as mild (AHI 5-14, n=27), moderate (AHI 15-29, n=22) ore severe (AHI [ge]30, n=31) OSAS. Continuous variables were expressed as means \pm SD, and categorical variables were expressed as frequencies. Comparisons were made by Student t-test, ANCOVA, or Chi² as appropriate. For multivariate analysis logistic regression analysis was used. A p value <0.05 was considered statistically significant. **Results:** When nt-OSA and non-OSA subjects were compared, differences were found in gender (female: 81.3 vs 96.7; p<0.05), age (43 \pm 10 vs 37 \pm 12 years; p<0.05), HDL-cholesterol (43 \pm 9 vs 47 \pm 11 mg/dl; p<0.05), HbA1C (5.5 \pm 1.1 vs 5.0 \pm 0.6%; p<0.01), fasting plasma glucose (109 \pm 14 vs 100 \pm 12 mg/dl; p<0.01), as well as in the prevalence of the MS (77,5 vs 50%; p<0,01). However, after adjusting for age and gender (ANCOVA), these differences became non significant. Furthermore, we did not find significant differences among groups when the OSAS severity was taken into account. Likewise, we did not find significant differences in the studied metabolic parameters between nt-OSAS and t-OSAS subjects, except for insulin-resistance (HOMA-R: nt-OSA: 6.9 \pm 4.2 vs. 12.2 \pm 7.7; P<0.05). **Conclusions:** We did not find significant differences in metabolic parameters between subjects with or without OSAS. Moreover, OSAS treatment with CPAP was not associated with an amelioration of the evaluated metabolic factors. Thus, our data suggest that OSAS does not represent an additional burden that exacerbates metabolic dysfunction in severely obese subjects.

Nothing to Disclose: GBAV, AJ, VPC, MMP, LFM, JFM, JV

Pub # P3-416

Session Information POSTER SESSION: CLINICAL - Obesity: Causes & Consequences (1:30 PM-3:30 PM)

Title Association between C-Reactive Protein, Carotid Intima-Media Thickness and P-Wave Dispersion in Obese Premenopausal Women

Author String U Ozuguz, G Ergun, S Isik, F Gokay, YA Tutuncu, G Akbaba, D Berker, S Guler
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Body

Objective: The aim of the present study was to evaluate P-wave duration and P dispersion (PWD) in obese women, and to investigate the relationship between P-wave measurements and high sensitive C-reactive protein (hsCRP), carotid intima-media thickness (CIMT) and echocardiographic variables.

Methods: The study population consisted of 44 obese premenopausal women and 30 normal weight control subjects. Anthropometric measurements [weight, height, waist circumference, waist-to-hip ratio, body mass index (BMI)] were recorded, fasting plasma glucose, insulin, homeostasis model assessment insulin resistance (HOMA-IR), hsCRP, lipid parameters and CIMT were measured. P-wave duration and P-wave dispersion were calculated on the 12-lead ECG. The echocardiographic parameters were assessed in detail in standard left lateral decubitus position.

Results: Maximum P-wave duration was longer (Pmax) and P dispersion was increased in obese women than the controls (105.2±14.3 vs 89.0±13.3 ms, P < 0.001 and 41.8±11.8 vs 28.5±9.3 ms, P < 0.001, respectively). Correlation analysis showed that there were positive correlations between PWD, and insulin, HOMA-IR, systolic blood pressure, diastolic blood pressure, hsCRP, CIMT, left atrial diameter (LAD), waist circumference, waist to hip ratio and BMI in obese group. In the linear regression analysis, there was significant association between only LAD and PWD ($\beta=4.73$, $p=0.024$).

Conclusion: Our data suggest that obesity affects P-wave dispersion and duration, and changes in P dispersion may be related to the subclinic inflammation and atherosclerosis as well as LAD.

Nothing to Disclose: UO, GE, SI, FG, YAT, GA, DB, SG

Pub # P3-417

Session Information POSTER SESSION: CLINICAL - Obesity: Causes & Consequences (1:30 PM-3:30 PM)

Title Association between Nonalcoholic Fatty Liver Disease with Metabolic Syndrome and Arterial Stiffness in Nondiabetic Men

Author String ES Kim, S Moon, J-H Han, CW Ahn, SA Kim, SH Beom, JS Yoo, M Cho, JS Park, BS Cha, KR Kim, HC Lee
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Body

Objectives
The independent relationship between nonalcoholic fatty liver disease (NAFLD) and cardiovascular disease (CVD) risk is still unclear, although several studies have suggested that NAFLD could play a role in atherosclerosis. We conducted this study to assess associations among NAFLD, metabolic syndrome, and arterial stiffness in nondiabetic men.

Methods
We recruited a total of 1025 nondiabetic men, aged 30 to 70 years, who visited our hospital for a health checkup. NAFLD was determined by ultrasonography, and arterial stiffness was evaluated by brachial-ankle pulse wave velocity (PWV).

Results
Of 1025 men, 285 had mild NAFLD, 234 had moderate-severe NAFLD. The prevalence of MetS increased according to normal, mild, overt NAFLD; 13.2%, 33.7%, 56.8%, respectively ($P < 0.001$). The risk for metabolic syndrome increased with NAFLD severity (mild NAFLD, odds ratio [OR] = 3.31, 95% confidence interval [CI] = 2.32-4.72; overt NAFLD, OR = 8.62, 95% CI = 5.99-12.42; P for trend < 0.001) after adjusting for age. In multivariate analysis, overt NAFLD was associated with high PWV (OR = 1.62; 95% CI = 1.02-2.57; $P = 0.042$) after adjustment for established risk factors and metabolic syndrome.

Conclusions
Overt NAFLD was closely associated with subclinical atherosclerosis independently of metabolic syndrome as well as conventional factors in nondiabetic men. These results suggest that life style modifications and reduction of combined CVD risks in subjects with NAFLD might have a beneficial effect on cardiovascular outcomes.

Nothing to Disclose: ESK, SM, J-HH, CWA, SAK, SHB, JSY, MC, JSP, BSC, KRK, HCL

Pub # P3-418

Session Information POSTER SESSION: CLINICAL - Obesity: Causes & Consequences (1:30 PM-3:30 PM)

Title Metabolic Syndrome and Arterial Stiffness in Korean Adults

Author String H Cho, G Yu, D Shin, J Park, H Jang, P Park, B Chun
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Sources of Research Support: Grant of the Korea Centers for Disease Control and Prevention (2005-E71011-00, 2006-E71009-00).

Nothing to Disclose: HC, GY, DS, JP, HJ, PP, BC

Pub # P3-419

Session Information POSTER SESSION: CLINICAL - Obesity: Causes & Consequences (1:30 PM-3:30 PM)

Title Adipocytokines as Pathogenetic Markers of Nonalcoholic Fatty Liver Disease in Patients with Metabolic Syndrome

Author String IO Kostitska
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Body

Introduction
Nonalcoholic fatty liver disease (NAFLD) often represents a cluster of the metabolic syndrome (MS) characterized by obesity, insulin resistance (IR), type 2 diabetes mellitus (DM), hypertriglyceridemia and hypertension. IR states are characterized by elevated production of several adipocytokines (leptin, resistin, visfatin, adiponectin, IL-6, TNF- α). Hyperleptinemia is considered a key factor in the development of IR underlying MS.

Purpose
The aim of this study was to investigate the role hyperleptinemia in pathogenesis of steatohepatitis in patients with MS.

Material
The study composed of 42 subjects with MS (mean age 48,4 \pm 8,2 years, duration of type 2 DM 6,8 \pm 3,9 years and body mass index 32,8 \pm 3,8 kg/m²).

Method
Fasting of leptin concentrations were measured by immunoassays. The diagnosis of steatohepatitis was based on biochemical markers and detection of fatty liver by ultrasonography. All subjects had negative markers of virus hepatitis, negative history of alcohol intake.

Results
The prevalence of NAFLD in patients with MS was 74%. Plasma levels of leptin were significantly higher in patients with steatohepatitis than in those with normal levels of leptin in subjects without NAFLD. Steatohepatitis was mild 53,8%, moderate in 29,8% and severe in 16,4% of cases. When the study was stratified as group A consist of patients with NAFLD mild and moderate steatohepatitis (83,6%), group B - severe steatohepatitis (16,4%) based on ultrasound evaluation and compared and group C - all subjects without NAFLD (26%). In group B elevated levels of leptin (p=0.005) were significantly higher than group A but in patients of C group hyperleptinemia were absence.

Conclusions
The prevalence of NAFLD is higher in patients with IR than in general population. Although hyperleptinemia is present in all patients with NAFLD than those without steatohepatitis.

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(2) Bertolani C, Fabio M, *Pathophysiology* 2008;15(2): 91-101
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Nothing to Disclose: IOK

Pub # P3-420

Session Information POSTER SESSION: CLINICAL - Obesity: Causes & Consequences (1:30 PM-3:30 PM)

Title Effects of Abdominal Visceral Fat and Age on Glucose-Induced Activation of the Corticotropic Axis in Healthy Men

Author String A Iranmanesh, DM Lawson, B Dunn, J Veldhuis
Salem VA Medical Center, Salem, VA; Mayo Clinic, Rochester, MN

Body Age and adiposity are variably reported to affect corticotropic function. In the present study, the dynamics of corticotropic responses to oral dextrose administration were assessed in 58 healthy men in the age range of 19-78 yrs and BMI of 20-39 Kg/m². Each subject was studied after an overnight fast on 2 separate occasions. During each session, blood was drawn at 10-min intervals starting at 0800-0900 hr for a total period of 6.5 hrs, with either 75 grams of dextrose or identical volume of water ingested orally after the 4th blood draw at min 30. Circulating concentrations of ACTH (pg/mL) and cortisol ([micro]g/dL) were measured in each blood sample, and their respective secretory properties were assessed by deconvolution analysis. ACTH/cortisol dose response was determined mathematically. Visceral fat area (VFA) was estimated by CT scan, and ranged from 12.4 to 410 cm². Data is presented as mean ±SE. As a group, oral intake of dextrose was associated with significant increases in the 6.5 hr sum of ACTH (857±51 vs 740±35: P=0.02) and cortisol (457±16 vs 410±13 P=0.0015). Deconvolution analysis of the 6.5 hr time series was significant for post-dextrose increases in pulsatile release of ACTH (111± 8.8 v 85±6 ng/L/6.5 h: P=0.009) and of cortisol (47±3 v 37±2.4 [micro] g/dL /6.5 h: P=0.001), with corresponding increases in the masses of ACTH (24± 2.4 vs 16±1.1 ng/L: P=0.001) and cortisol (12±0.9 vs 9.1±0.6 [micro]g/dL: P=0.004) secreted per burst. Post-glucose increment in pulsatile cortisol release was closely associated with matching increase in pulsatile ACTH secretion (R=0.57, P<0.001). While ACTH/cortisol dose responses were independent of VFA and age, VFA was positively associated with incremental effect of glucose on cortisol secretory burst mass (R=0.32, P=0.019). Unexpectedly, the negative effect of age on pulsatile ACTH and mass of ACTH secreted per burst (control day) was abolished after dextrose ingestion. In summary, the results of this study demonstrate corticotropic-axis stimulatory effect of oral glucose, characterized by amplified pulsatile secretion with increased mass of ACTH and cortisol released per burst. The positive effect of visceral fat on incremental pulsatile release of cortisol could be suggestive of increased corticotropic responses in obesity, which warrants future investigation.

Nothing to Disclose: AI, DML, BD, JV

Pub # P3-421

Session Information POSTER SESSION: CLINICAL - Obesity: Causes & Consequences (1:30 PM-3:30 PM)

Title Prevalence of Abnormal Metabolic Parameters in Overweight Non-Diabetic Children

Author String MA Siddiqui, A Ahmad, M Gupta, N Gupta, SK Wangnoo
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Body

Background:
Increasing rates of overweight and obesity has reached epidemic proportions in developed countries and is rapidly increasing in many middle-income and less-developed countries. Childhood obesity increases the risk of adult obesity as well as chronic health problems such as type 2 diabetes, hypertension and cardiovascular disease. The purpose of this study was to identify the prevalence of abnormal metabolic parameters in overweight non-diabetic children visiting a tertiary hospital.

Method:
We included 59 overweight, aged 8-18 years, (BMI \geq 85th percentile for age and sex) but otherwise healthy children (M:39, F:20), who underwent assessment of glucose tolerance (fasting and post-75 gm 2 hr. OGTT), insulin levels, lipids, serum transaminases, 25(OH)vitamin-D levels, hsCRP and height and weight measurements and BMI calculation (Asian-Indian criteria). Body composition was measured by dual energy X-ray absorptiometry and pubertal staging was done with Tanner staging.
ANCOVA, adjusted for age, sex, Tanner stage (prepubertal and pubertal), and percent body fat (measured by DEXA), was used to compare metabolic variables between the quartiles of glucose and insulin groups.

Results:
Tanner stage was 22.9% prepubertal, 77.1% pubertal; mean BMI 29.1 \pm 5.8 kg/m²; BMI percentile 92.2 \pm 2.0; and percent body fat 43.2 \pm 7.5%. Although the plasma glucose values were within normal limits, with increasing quartiles of plasma glucose, plasma insulin levels also demonstrated an increasing trend. Systolic and diastolic blood pressure and triglycerides increased significantly with increasing quartiles of fasting plasma glucose and insulin. Elevated hsCRP and serum transaminases, low levels of 25(OH)vitamin-D and HDL were seen with increasing quartiles of 2-h plasma glucose and insulin.

Conclusions:
Even with plasma glucose levels within the normal range, overweight non-diabetic children demonstrate metabolic derangements that favour a future risk of development of cardiovascular damage.

Nothing to Disclose: MAS, AA, MG, NG, SKW

Pub # P3-422

Session Information POSTER SESSION: CLINICAL - Obesity: Causes & Consequences (1:30 PM-3:30 PM)

Title The Prevalence of Metabolic Syndrome in Obese Adolescents of South Bronx: Comparison of IDF and NCEP Criteria

Author String A Badheka, R Senguttuvan, S Dave-Sharma
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Body

Objective:
The purpose of our study was to compare the prevalence of Metabolic syndrome (M.S) using proposed National Cholesterol Education Program (NCEP)¹ and International Diabetes Federation (IDF)² criteria in obese patients of community hospital in South Bronx.

Background:
M.S. diagnostic criteria are not well characterized in pediatric population. There is a lack of agreement to its standard definition and cut-off points, though most definitions include abdominal obesity, high blood pressure, glucose and lipid metabolism abnormalities. The National Cholesterol Education Program (NCEP Adult Treatment Panel III) proposed well accepted criteria¹ for pediatric M.S. which are based on age related percentiles that are time consuming and create difficulty of its use in clinical setting. International Diabetic Federation (IDF) proposed clinically simpler definition of M.S.² which is based on absolute cut off values. The purpose of our study was to compare the prevalence of M.S. using proposed NCEP and IDF criteria in obese patients of community hospital in South Bronx.

Methods:
We applied the metabolic syndrome diagnostic criteria proposed by NCEP and IDF on 147 adolescents (85 males and 62 females) between 10 to 19 years. In this prospective study; patients' height, weight, waist circumference and blood pressure were assessed. Body Mass Index(BMI) calculated by using standard formula. Body height, weight and BMI plotted on standard CDC growth chart for age. Fasting serum concentrations of HDL-C, triglycerides, glucose and insulin levels were measured and insulin sensitivity was calculated by use of the quantitative HOMA insulin sensitivity check index (3).

Results:
Prevalence of M.S. by NCEP ATP III definition for age in our male pediatric population is 29.26% which is equivalent to IDF definition prevalence of 28.04%. However, young female M.S. prevalence is considerably high by IDF definition 33.33 % compared to NCEP ATP III definition prevalence of 30.16%.

Conclusion:
IDF criteria for adolescent are not only easier in clinical setting as well shows almost equal sensitivity to NCEP in male population and better sensitivity in female population.

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(2) Zimmet Pbet al., The metabolic syndrome in children and adolescents. Lancet. 2007;369(9579):2059-2061

(3) Matthews, D.R., Hosker, J.P., Rudenski, A.S., Naylor, B.A., Treacher, D.F. and Turner, R.C. (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28, 412-419

Nothing to Disclose: AB, RS, SD-S

Pub # P3-423

Session Information POSTER SESSION: CLINICAL - Obesity: Causes & Consequences (1:30 PM-3:30 PM)

Title Childhood Trajectories of BMI Z-Score Affect Adult Glucose Regulation

Author String MS Thearle, S Votruba, J Krakoff
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Body Differing patterns of childhood growth may exist within a population and the path to adult BMI may influence risk for glucose dysregulation. Latent class trajectory analysis was used to evaluate patterns of change in BMI z-scores in 1861 children of at least 50% Pima heritage with data from 4 or more visits between the ages of 5 and 20 years during a longitudinal study of health where individuals were invited for exams, including measures of height, weight and a 75 g oral glucose tolerance test, every 2 years. Nine distinct trajectories of BMI z-score over the 15 years were found, best described as: (1) a consistent z-score of -1 (2.8%), (2) a steady rise in z-score from -1 to 0 (3.7%), (3) a consistent z-score of 0 (7.8%), (4) a linear rise in z-score from 0 to 1 (12.6%), (5) an early rapid increase from an initial z-score of 0 to stabilization at 1.5 (11.4%), (6) a z-score that declined from 1 to 0.5 after age 13 (9.9%), (7) an early rise in z-score from 1 to 1.5 before stabilization after age 12 (20.2%), (8) a consistent z-score of 2 (18.2%), and (9) a consistent z-score of 2.5 (13.4%). Offspring of diabetic mothers (ODM) were disproportionately represented in trajectories 8 and 9 such that 57% of ODM were in these 2 trajectories ($\chi^2 = 28$; $p < 0.001$). In linear regression models, childhood group was a predictor of fasting and 2 hour glucose in the 1245 adults (>20 yrs) with available data, independent of age, sex, degree of Pima heritage and concurrent adult BMI. In logistic regression analysis, the odds ratios (OR) for development of type 2 diabetes compared to individuals in group 3 was significantly elevated for groups 5 (OR=3.2; $p=0.01$), 7 (OR 3.6; $p<0.001$), 8 (OR=4.7; $p<0.001$) and 9 (OR=8.0; $p<0.0001$). A subset of 324 individuals participated as adults in a study on the determinants of type 2 diabetes. Childhood group, adjusted for age, sex and ethnicity, was a predictor of adult %fat ($p<0.001$; partial $r^2 = 0.28$). Ninety-five full heritage individuals with normal glucose regulation had measures for insulin action (M) as determined by a euglycemic-hyperinsulinemic clamp and for acute insulin release (AIR) measured after a 25g IV glucose bolus. Childhood group was not a predictor of AIR ($p=0.4$) but was a determinant of M ($p=0.02$; partial $r^2 = 0.13$), independent of age, sex and adult %fat. In conclusion, individuals can be classified into different categories of childhood BMI z-score pattern. Such categories may be useful as predictors of adult risk.

Sources of Research Support: Intramural Research Program of the NIH / NIDDK.

Nothing to Disclose: MST, SV, JK

Pub # P3-424

Session Information POSTER SESSION: CLINICAL - Obesity: Causes & Consequences (1:30 PM-3:30 PM)

Title Obesity in Adolescent Population of South Bronx and Its Influence on Comorbidities Leading to ER Visits and Hospitalization

Author String G Paudel, S Vernekar, S Jacob, D Vasquez, R Senguttuvan, L Blanca, D Alvarez, S Khanna, M Waseem, S Dave-Sharma
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Body

Objective:
To determine the effect of obesity in severity of disease measuring frequency of emergency room (ER) visits, frequency of hospitalizations and duration of hospital stay.

Method:
A retrospective chart review of hospitalized patients of 10-19 years age in the year 2009 was done. Obese, overweight and normal weight population was compared for age, gender, race, hospital stay, admitting diagnosis, co-morbidities, number of ED visits and hospitalizations. Statistical analysis was performed using SPSS 16.

Result:
Among 724 patients, 50% were male and mean age was 14.5 years. Obese constituted 23.5% of the hospitalized population which is higher than the general prevalence of adolescent obesity in New York (11 to 17%). There was statistically significant difference between mean ED visits in obese (5.5), normal weight (4.3) and underweight (1.6) population ($p < 0.001$). No statistical difference was seen in hospital stay and number of hospitalizations in different BMI categories. Diagnosis of status asthmaticus and soft tissue infection altogether represented 45.3% in obese, 26.7% in normal weight and 12.5% in underweight patient population. Statistically significant Higher ED visits were found in obese patients with status asthmaticus (7.8 compared to 6.7 for normal, 2.3 for underweight) soft tissue infections (4.6 compared to 3.4 in normal) and renal diseases (6.0 compared to 2.8 in normal). Asthma was more prevalent co-morbidity in hospitalized obese than normal weight patients (46.5% vs 32.2%).

Conclusion:
Obesity is associated with higher rate of co-morbidities and ED visits. The principal diagnoses for frequent ED visits amongst obese individuals are asthma, soft tissue infections, and renal diseases.

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2. Obesity (emedicine web article)

Author: Gabriel I Uwaifo, MBBS, Clinical and Research Attending, Assistant Professor of edicine and Endocrinology, MedStar Clinical Research Center, MedStar Research Institute and Washington Hospital Center

Coauthor(s): Elif Arioglu, MD, Assistant Professor of Medicine, Division of Endocrinology and Metabolism, University of Michigan

Updated on May 21 2009

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Nothing to Disclose: GP, SV, SJ, DV, RS, LB, DA, SK, MW, SD-S

Pub # P3-425

Session Information POSTER SESSION: CLINICAL - Obesity: Causes & Consequences (1:30 PM-3:30 PM)

Title Testosterone Concentration in Obese Young Males

Author String M Mogri, S Dhindsa, H Ghanim, P Dandona, T Quattrin
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Body AIM:Obesity in adult males is associated with hypogonadotropic hypogonadism. In children, body mass index (BMI) has known to affect the age of initiation of puberty in both males and females. The objective of our study was to look at the effect of obesity on plasma testosterone concentrations in post pubertal male children
RESEARCH DESIGN AND METHODS:This was a cross-sectional case control study performed at Women and Children's Hospital of Buffalo, Endocrine and Diabetes Center. Blood samples were obtained between 8 and 10 am from 19 obese (BMI \geq 97% for age) and 11 lean (BMI < 85% for age) males between the ages 14-21 years with Tanner staging \geq 4. Total testosterone (TT), sex hormone binding globulin (SHBG) and C-reactive protein (CRP) levels were measured. TT was measured by LC-MS/MS, SHBG by an immunometric assay and CRP by ELISA. Free testosterone (cFT) was calculated from TT, SHBG and serum albumin. RESULTS: Obese males had a significantly lower TT, cFT and SHBG concentrations when compared to lean males: TT: 296 \pm 131 ng/dl vs 536 \pm 146 ng/dl, p<0.001; cFT: 7.5 \pm 3 vs 10.6 \pm 3.9 ng/dl; p=0.04; and SHBG 22 \pm 13 vs 46 \pm 25 nmol/l; p=0.01. After controlling for age and Tanner staging, the concentrations for total and free testosterone continued to be significantly lower in the obese males compared to the lean males. The CRP concentrations were higher in obese males when compared to the lean (2.2 \pm 1.9 vs 1.0 \pm 2.3 mg/l; p=0.001). TT (r=-0.67; p<0.001), cFT (r=-0.40; p=0.04) and SHBG (r=-0.56; p=0.002) were inversely related to BMI. CRP concentrations were inversely related to TT(r=-0.39; p=0.04) and SHBG(r=-0.40, p=0.04) and positively to BMI(r=0.65; p<0.001). CONCLUSION: Young obese post pubertal males have significantly lower TT, cFT and SHBG concentrations than those with normal BMI. The inverse correlation of these indices with CRP also suggests that these patients with lower T concentrations may be at a greater cardiovascular risk. Obese young men need to be screened for hypogonadism, systemic inflammation and potential cardiovascular risk.

Nothing to Disclose: MM, SD, HG, PD, TQ

Pub # P3-426

Session Information POSTER SESSION: CLINICAL - Obesity: Causes & Consequences (1:30 PM-3:30 PM)

Title Serum IGFBP-1 Concentration and Its Relation to Insulin, Leptin Levels, Age, Duration of Sleep and Breastfeeding in Obese Children

Author String L Pisarchyk, L Ibarra, ME Garay Sevilla, EL Perez-Luque, JM Malacara
University of Guanajuato, Leon, Mexico

Body

Background.
Emerging childhood obesity represents a risk factor for DM2, hypertension, cardiovascular disease and metabolic syndrome. IGFBP-1 has been reported as a marker of hepatic insulin resistance and to be associated with the components of the metabolic syndrome. Therefore, the study of the metabolic actions of IGFBP-1 in obesity is an important subject of study.

Material and methods.
We studied 182 obese prepuberal children identified in school in the city of Leon, Mexico, aged 6-11 diagnosed according to current international criteria. Anthropometric measurements were registered, and acantosis nigricans was assessed. A 10/h fasting blood sample was obtained to measure lipid profile, glucose, leptin, insulin measured by RIA, and IGFBP-1 levels measured by ELISA.

Results.
Ninety boys and 92 girls with a mean age of 9.1 ± 1.4 years were studied. They had a BMI of 27 ± 3.6 , HOMA-IR 5.8 ± 3.3 , insulin levels 27.0 ± 12.7 IU/L, IGFBP-1 5.0 ± 4.4 ng/ml. Acantosis nigricans was found in 102 children and absent in 54 children. Serum IGFBP-1 was inversely associated with BMI ($r = -0.30$, $P < 0.0001$). In a multiple regression procedure serum IGFBP-1 concentration correlated negatively with insulin ($t = -4.87$, $p < .00002$), leptin ($t = -2.25$, $p < 0.012$), age ($t = -3.14$, $p < 0.0019$), duration of breastfeeding ($t = -2.77$, $p < 0.006$) and positively with duration of sleeping ($t = 2.28$, $p < 0.023$).

Conclusions
These results confirm the negative association of IGFBP-1 with insulin resistance, but show other independent associations. IGFBP-1 shows also negative association with age, duration of breastfeeding, and with leptin. Those factors may indicate metabolic influences not yet described.

Sources of Research Support: Grant SEP-CONACYT 1203.

Nothing to Disclose: LP, LI, MEGS, ELP-L, JMM

Pub # P3-427
Session Information POSTER SESSION: CLINICAL - Obesity: Causes & Consequences (1:30 PM-3:30 PM)
Title Serum Leptin Concentrations Are Not Associated with Cardiovascular Disease and Do Not Predict Mortality in Chronic Hemodialysis Patients
Author String JJ Diez, M Bossola, MJ Fernandez-Reyes, E Di Stasio, L Tazza, G Luciani, R Codoceo, P Iglesias, A Rodriguez, E Gonzalez, R Selgas
Hospital Ramón y Cajal, Madrid, Spain; Istituto di Clinica Chirurgica, Universit[agrave] Cattolica del Sacro Cuore, Roma, Italy; Hospital General, Segovia, Spain; Hospital Universitario La Paz, IdiPAZ, Madrid, Spain; Hospital Universitario La Paz, IdiPAZ, Madrid, Spain

Body **Background.** End-stage renal disease (ESRD) patients exhibit high serum levels of leptin, a prothrombotic and pro-atherogenic adipocytokine (1,2). Several prospective studies have reported an association between elevated serum leptin concentrations and the risk of future cardiovascular events in the general population (3,4), and it has been suggested that high leptin levels may contribute to the increased cardiovascular risk in hemodialysis (HD) patients.
Objective. Our aim was to evaluate the relationship between serum leptin concentrations and the leptin/body mass index (BMI) ratio with prevalent cardiovascular disease (CVD), and their influence on all-cause and CVD-related mortality in ESRD patients undergoing maintenance HD.
Methods. We performed a prospective observational study in 118 stable HD patients (50 women, median [interquartile range] age, 65.1 [54.7-72.2] years) with a median follow-up time of 24.7 (15.7-68.0) months. All patients had baseline measurement of serum leptin concentrations. We analysed the relationship of serum leptin and the leptin/BMI ratio with the presence of prevalent CVD and ischemic heart disease. Relationships between leptin and all-cause and CVD mortality were studied by means of survival analysis and Cox regression analysis.
Results. The leptin/BMI ratio was similar in patients with and without CVD at baseline (0.65 [0.29-2.23] vs. 0.68 [0.29-1.49], respectively, NS), as well as it occurred in patients with and without coronary heart disease (0.73 [0.24-2.71] vs. 0.67 [0.28-1.47], respectively, NS). Multiple logistic regression analysis showed that there was not an independent association between leptin/BMI ratio and prevalent CVD. During follow-up time, 52 (44.1%) patients died, 27 (22.8%) of them as a result of CVD. Mean survival time for all-cause mortality and for CVD mortality was similar in patients classified according to values of serum leptin concentrations and the leptin/BMI ratio. Cox proportional multivariate regression analysis showed that there were no significant relationships between leptin levels or the leptin/BMI ratio and all-cause and CVD-related mortality.
Conclusion. These results suggest that, in stable HD patients, serum leptin concentrations and the leptin/BMI ratio are not related with prevalent CVD or coronary artery disease. Moreover, leptin/BMI ratio is not a risk factor for mortality in these patients.

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