Nuclear Magnetic Resonance Studies of Hepatic Glucose Metabolism in Humans

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ABSTRACT

Nuclear magnetic resonance (NMR) spectroscopy has made noninvasive and repetitive measurements of human hepatic glycogen concentrations possible. Monitoring of liver glycogen in real-time mode has demonstrated that glycogen concentrations decrease linearly and that net hepatic glycogenolysis contributes only about 50 percent to glucose production during the early period of a fast. Following a mixed meal, hepatic glycogen represents approximately 20 percent of the ingested carbohydrates, while only about 10 percent of an intravenous glucose load is retained by the liver as glycogen. During mixed-meal ingestion, poorly controlled type 1 diabetic patients synthesize only about 30 percent of the glycogen stored in livers of nondiabetic humans studied under similar conditions. Reduced net glycogen synthesis can be improved but not normalized by short-term, intensified insulin treatment. A decreased increment in liver glycogen content following meals was also found in patients with maturity-onset diabetes of the young due to glucokinase mutations (MODY-2). In patients with poorly controlled type 2 diabetes, fasting hyperglycemia can be attributed mainly to increased rates of endogenous glucose production, which was found by ^{13}C NMR to be due to increased rates of gluconeogenesis. Metformin treatment improved fasting hyperglycemia in these patients through a reduction in hepatic glucose production, which could be attributed to a decrease in gluconeogenesis. In conclusion, NMR spectroscopy has provided new insights into the pathogenesis of hyperglycemia in type 1, type 2, and MODY diabetes and offers the potential of providing new insights into the mechanism of action of novel antidabetic therapies.

I. Background

The pivotal role of the liver for maintaining normal glucose homeostasis resides in its ability to both deposit large amounts of glucose in the form of glycogen and to rapidly release glucose from its glycogen stores into the circulation by glycogenolysis. In addition, the liver – and, to a minor extent, the kidney — take up lactate, glycerol, and amino acids and convert these substrates to glucose by gluconeogenesis, which also contributes to glucose production. While hepatic glucose metabolism has been extensively investigated *in vitro,* many of its features remain unclear in humans.

Prior to the introduction of *in vivo* NMR spectroscopy (Rothman *et al.,* 1991a), studies in humans to estimate hepatic glucose metabolism were limited to either invasive or indirect approaches. Liver biopsies allow direct quantification of glycogen concentration but are invasive and cannot easily be repeated within one experiment (Behringer and Thaler, 1964; Nilsson *et al.,* 1973,1974). Hepatic venous catheterization has been used to measure net splanchnic uptake of gluconeogenic substrates, thereby providing an estimate of hepatic gluconeogenesis (Wahren *et al.,* 1972). Because this method does not account for intrahepatic gluconeogenic substrates or uptake of lactate and amino acids formed within the splanchnic bed (Björkman *et al.,* 1990), it will underestimate hepatic gluconeogenesis. In addition, incorporation of labeled carbons into glucose using isotope dilution techniques (Consoli *et al.,* 1987) does not permit quantification of gluconeogenesis (Landau, 1999; Landau *et al.,* 1995,1996). Finally, mass isotopomer analysis following [U-$^{13}C_6$]glucose (Tayek and Katz, 1997) or ^{13}C glycerol administration (Hellerstein *et al.,* 1997) will underestimate gluconeogenesis, as was reviewed recently (Kelleher, 1999; Radziuk and Lee, 1999; Landau, 1999; Previs *et al.,* 1999).

Carbon-13 nuclear magnetic resonance (^{13}C NMR) spectroscopy has made noninvasive and repetitive measurements of hepatic glycogen concentrations in real-time mode possible, permitting the calculation of rates of net hepatic glycogen synthesis and glycogenolysis *in vivo.* Furthermore, using ^{13}C-labeled glucose pulse-^{12}C glucose chase experiments, rates of hepatic glycogen turnover can be assessed. Previous reviews have discussed other aspects of this topic (Alger *et al.,* 1981; Shulman *et al.,* 1984; Rothman *et al.,* 1991b; Taylor and Shulman, 1994; Beckmann, 1995; Roden and Shulman, 1999).

II. Basic Aspects of *in Vivo* NMR Spectroscopy

The basic principles of *in vivo* NMR spectroscopy have been described previously in detail (Gadian *et al.,* 1982; Taylor and Shulman, 1994; Roden and Shulman, 1999). Briefly, some nuclei possess magnetic properties — the magnetic moment or "spin." Within a strong, static magnetic field (B_0 field) of a NMR spectrometer, the nuclei spin around their own axis with a characteristic frequency in order to align with or against the magnetic field. Stimulation of nuclei by an additional oscillating magnetic field (B_1 field) at their frequency of processing causes these nuclei to swing out of alignment. Relaxation of the nuclei to the low-energetic state causes emission of radiowaves that are detected by a receiver coil. Under experimental conditions, resonant waves from various nuclei are superimposed, giving the picture of oscillating amplitudes in a display of intensities vs. time (free induction decay, FID). Fourier transformation translates the FID into a display of signal intensities vs. frequencies. The peak frequency is characteristic for a certain nucleus/compound; the area under that peak corresponds to

its tissue concentration. That concentration can be converted into molar terms by comparison with the area under the peak of a phantom containing a defined concentration of that nucleus/compound (i.e., external standard) or by comparison with the resonance of an intrinsic compound with known concentration (i.e., internal standard). Several measures have been developed to improve the signal-to-noise ratio of NMR spectroscopy: 1) using special ^{1}H (decoupling) coils to eliminate the interaction between ^{13}C and its surrounding protons; 2) shimming procedures to increase the homogeneity of the static magnetic field by changing currents; 3) increasing the field strength of whole-body NMR spectrometers; 4) adding spectra prior to Fourier transformation; 5) placing surface coils tightly over the region of interest, to ensure homogenous tissue filling; and 6) selecting the pulse angle to suppress signals from other tissues such as the subcutaneous fat layer.

Despite a low natural abundance of 1.1%, ^{13}C can be used to measure hepatic glycogen (Rothman *et al.*, 1991a) and triglyceride content (Petersen *et al.*, 1996a), which typically occur in concentrations over 50 mmol/l. Since the resonance of ^{13}C in the C1 position of glycogen is clearly resolved at 100.5 ppm and all ^{13}C signals from glycogen are detected by ^{13}C NMR spectroscopy (Shulman *et al.*, 1988; Gruetter *et al.*, 1994), the peak integral of C1 glycogen corresponds to a defined glycogen concentration (Figure 1). Validation of ^{13}C NMR spectroscopic measurements revealed an excellent agreement ($r = 0.95$) with classical chemical determination of glycogen over a broad range of glycogen concentrations (Shulman *et al.*, 1988; Borgs *et al.*, 1993), demonstrating that liver (Sillerud and Shulman, 1983; Shalwitz *et al.*, 1987; Gruetter *et al.*, 1994) and muscle (Gruetter *et al.*, 1991; Taylor *et al.*, 1992; Roussel *et al.*, 1997) glycogen is essentially 100 percent visible by ^{13}C NMR. NMR spectroscopy is even more precise than the biopsy measurements. Coefficients of variation between multiple measurements in the same subject are 7 percent in liver (Rothman *et al.*, 1991a).

In addition to quantification of natural abundance ^{13}C glycogen, ^{13}C NMR spectroscopy can be applied to trace ^{13}C incorporation into glycogen during infusion or ingestion of [1-^{13}C]-enriched glucose (10-99 percent), which increases the sensitivity of the method by up to 100-fold. Sequential infusions of ^{13}C-enriched and unlabeled glucose (^{13}C pulse-^{12}C chase experiments) (Shulman *et al.*, 1988) allow assessment of rates of glycogen synthesis and simultaneous glycogenolysis in humans (Magnusson *et al.*, 1994; Roden *et al.*, 1996; Petersen *et al.*, 1998). In these experiments, the increment in total hepatic glycogen over time during infusion of [1-^{13}C] glucose gives the flux through glycogen synthase. To obtain an estimate of glycogenolysis, the change of the [1-^{13}C]glycogen concentration is compared with the predicted increment, assuming constant flux through glycogen synthase and no glycogen breakdown. From the difference between predicted and observed glycogen concentrations, one can assess a minimum estimate of simultaneous glycogenolysis (i.e., only the glycogen that is broken down

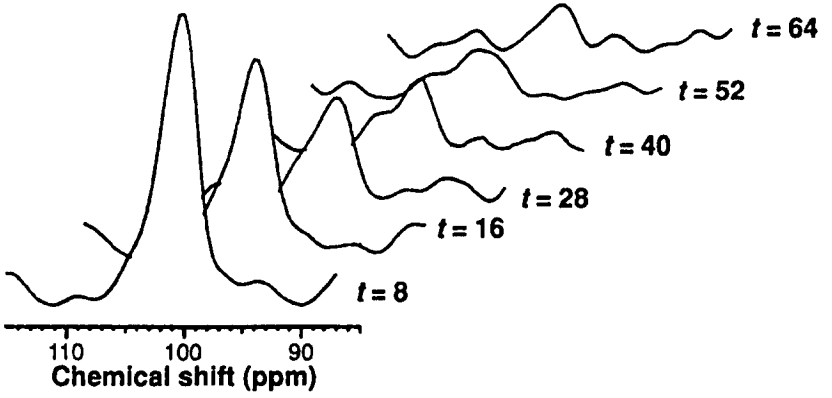


FIG. 1. Staggered plot of ^{13}C NMR spectra of the C1 position of natural abundance hepatic glycogen in one subject at indicated hours of fasting. [Reprinted with permission from Rothman, D.L., Magnusson, I., Katz, L.D., Shulman, R.G., and Shulman, G.I. *Science* **254**, 573-576, 1991. Copyright 1991 American Association for the Advancement of Science.]

and escapes the hexose-1-phosphate pool). The ratio of glycogen breakdown to glycogen synthesis yields relative rates of glycogen turnover (Villar-Palasi and Larner, 1960).

The liver offers specific anatomical advantages to study glycogen metabolism by NMR spectroscopy. Its volume (~ 1500 cm^3) and proximity to the body surface allow homogenous tissue filling under the surface coil. Hepatocytes, which primarily account for glycogen metabolism, represent approximately 80 percent of parenchymal volume of the liver. In addition, glucose metabolism – and, in particular, the relative contribution of the pathways of glycogen synthesis — is similar across the liver acinus (Cline and Shulman, 1991,1995), although hepatocytes from periportal and perivenous zones of the liver acinus may differ in their enzyme pattern and subcellular structures (Jungermann and Katz, 1989).

III. Studies on Liver Glycogen Metabolism in Humans

A. PHYSIOLOGIC REGULATION OF HEPATIC GLYCOGEN METABOLISM

1. The Postabsorptive State

In the postabsorptive (i.e., fasting) state, both glycogenolysis and gluconeogenesis are required to maintain normal rates of endogenous glucose production. However, prior to NMR techniques, the relative contributions of gluconeogenesis and glycogenolysis to glucose production in humans were unknown.

Employing ^{13}C NMR spectroscopy in combination with magnetic resonance imaging (MRI) of liver volume and infusion of [3-^{3}H]glucose to determine glucose production, the time course of hepatic glycogen concentrations was followed over 68 hours of fasting (Rothman *et al.,* 1991a) (Figure 1). During the first 22 hours of fasting, liver glycogen decreased nearly linearly, from 396 ± 29 mmol/l at 4 hours after a modest-sized meal of 650 kcal to 251 ± 30 mmol/l at 15 hours. This fasting concentration agreed with that of approximately 270 mmol/l obtained by needle biopsy (Nilsson and Hultman, 1973) and was later confirmed by another group employing ^{13}C NMR spectroscopy (Fried *et al.,* 1996). Net rates of glycogenolysis were calculated to be 4.3 ± 0.6 $\mu mol.kg^{-1}.min^{-1}$ (0.20 ± 0.02 $\mu mol.l^{-1}.min^{-1}$), which surprisingly accounted for only 36 percent of whole-body glucose production (Figure 2). Thereafter, net glycogenolysis decreased to 1.7 ± 0.5 $\mu mol.kg^{-1}.min^{-1}$ between 22 hours and 46 hours and to 0.3 ± 0.6 $\mu mol.kg^{-1}.min^{-1}$ between 46 hours and 64 hours. After 64 hours, liver glycogen content and volume had decreased by 83 and 23 percent, respectively, and gluconeogenesis was almost exclusively (i.e., 96 percent) responsible for endogenous glucose production. The relatively large contribution of gluconeogenesis (64 ± 5 percent) to glucose production, even after 22 hours of fasting, is in contrast to earlier studies reporting less than 38 percent after a fast of 12-14 hours (Nilsson and Hultman, 1973; Wahren *et al.,* 1972; Consoli *et al.,* 1987). In order to estimate the contribution of glycogenolysis to glucose production during the earliest hours of an overnight fast, net glycogenolysis was followed 5-12 hours after a large, 1000-kcal meal (Petersen *et al.,* 1996b). Whole-body glucose production was determined using [6,6-^{2}H]glucose, which yields a minimum estimate of glucose production (Miyoshi *et al.,* 1988). Even under these conditions, net hepatic glycogenolysis (5.8 ± 0.8 $\mu mol.kg^{-1}.min^{-1}$) contributed only 45 ± 6 percent to whole-body glucose production. This result implies that gluconeogenesis typically contributes about 50 percent to the rate of whole-body glucose metabolism during the early hours following a mixed meal (Figure 2) (Petersen *et al.,* 1996b). This provocative results was recently confirmed using a novel D_2O method to directly assess gluconeogenesis (Landau *et al.,* 1996).

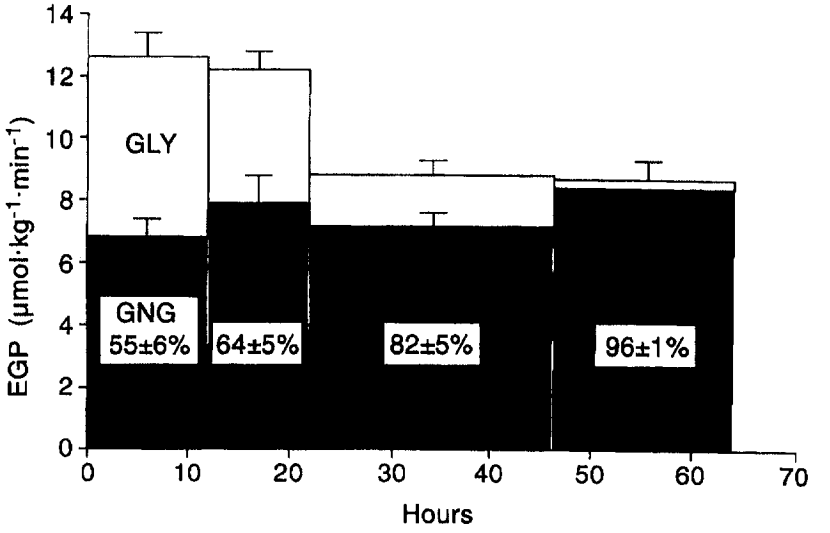


FIG. 2. Percent contribution of gluconeogenesis (GNG) and glycogenolysis (GLY) to glucose production during fasting. [Data from Rothman, D.L., Magnusson, I., Katz, L.D., Shulman, R.G., and Shulman, G.I. *Science* **254,** 573-576, 1991; and Petersen, K.F., Price, T., Cline, G.W., Rothman, D.L., and Shulman, G.I. *Am. J. Physiol.* **270,** E186-E191, 1996.].

2. The Postprandial State

Following an oral glucose load, hepatic glucose production is suppressed within 30 minutes (Taylor *et al.,* 1996) and the liver takes up glucose to replenish hepatic glycogen stores. Typically, studies on the regulation of hepatic glucose removal were limited to measuring splanchnic glucose uptake by isotopic methods or hepatic vein catheterization (Davies *et al.,* 1994; DeFronzo, 1997) but could not determine the fate of glucose within the liver.

When liver glycogen concentrations were monitored with ^{13}C NMR following a mixed meal, liver glycogen increased from 207 ± 22 mmol/l after an overnight fast, to peak values of 316 ± 19 mmol/l at 5 hours, giving a net rate of glycogen synthesis of 0.34 $\mu mol.l^{-1}.min^{-1}$ (Taylor *et al.,* 1996) (Figure 3). From these data, Taylor and coworkers (1996) calculated that 28.3 ± 3.7 g glucose were deposited as glycogen in the liver, which represents approximately 19 percent of the carbohydrate contents in the meal. The data are consistent with estimates of about 25 percent obtained after an oral 150-g glucose load by double tracer studies (Radziuk *et al.,* 1978), splanchnic balance techniques (Katz *et al.,* 1983), and a different NMR study (Beckmann *et al.,* 1993). After the peak, liver glycogen concentrations decreased linearly, at a rate of 0.26 $mmol.l\text{-}^{-1}.min^{-1}$, in parallel with the fall of the insulin/glucagon ratio.

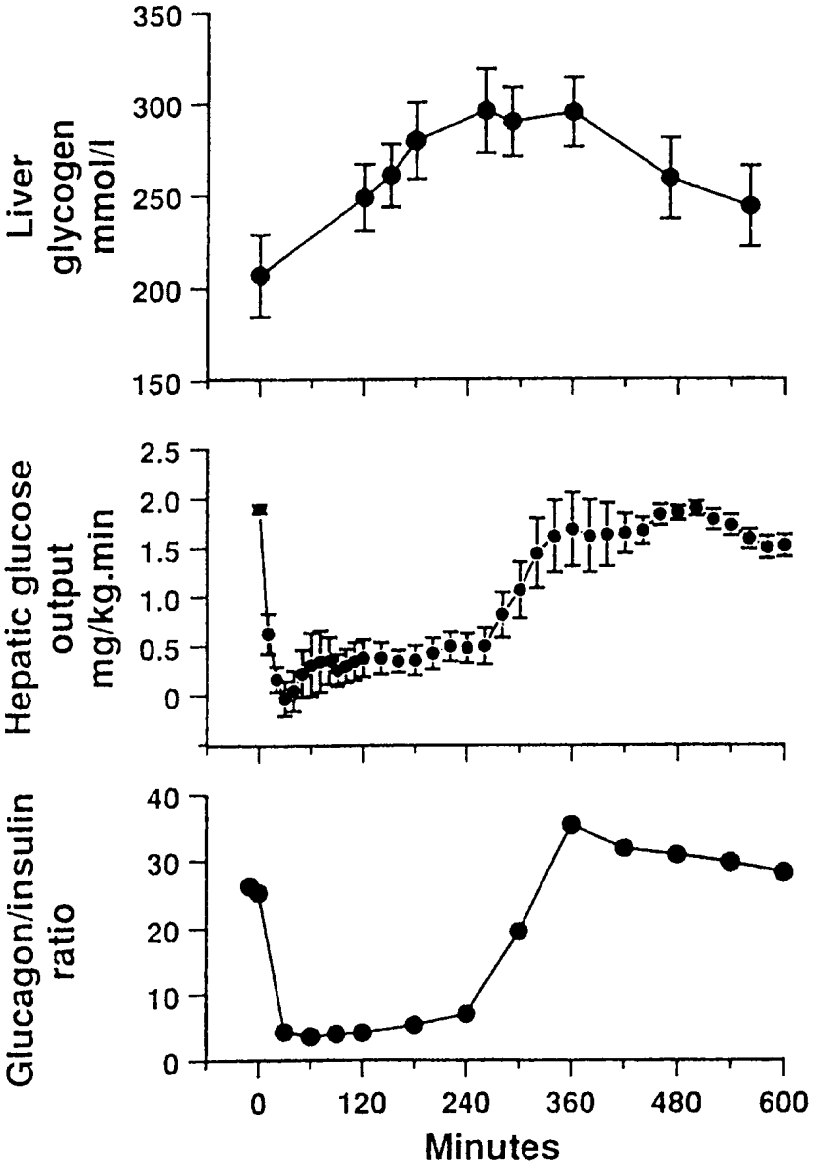


FIG. 3. Changes in liver glycogen concentration, endogenous glucose production, and plasma glucagon/insulin ratio after a mixed meal ingested at zero time. [Reprinted with permission from Taylor, R., Magnusson, I., Rothman, D.L., Cline, G.W., Caumo, A., Cobelli, C., and Shulman, G.I. *J. Clin. Invest.* **97**, 126-132, 1996.]

During an intravenous glucose load enriched with [1-^{13}C]glucose, glycogen synthetic rate was 0.49 ± 0.10 μmol.l^{-1}.min^{-1} (Cline *et al.*, 1994). Net hepatic glycogen synthesis (~ 0.34 μmol.l^{-1}.min^{-1}) accounted for around 12 percent of whole-body nonoxidative glucose metabolism. Using ^{13}C NMR spectroscopy, another group estimated that glucose uptake by the liver could account for up to 10 percent of the infused glucose under similar conditions of hyperglycemia (~ 9 mmol/l vs. 10.5 mmol/l) but lower plasma concentrations of insulin (~ 120 pmol/l vs. 400 pmol/l) (Beckmann *et al.*, 1993).

In order to examine changes in hepatic glycogen content under conditions of normal eating behavior, nondiabetic subjects ingested three mixed meals over the course of a day, with liver glycogen levels monitored following each meal (Hwang *et al.*, 1995). Liver glycogen stores increased from 274 ± 11 mmol/l, reached a peak just before the next meal, and were maximal at 4 hours after dinner (420 ± 19 mmol/l). This indicates negligible net hepatic glycogenolysis, when meals are ingested 5 hours apart during the course of a day. Thus, glucose absorption from meals and gluconeogenesis account for the major part of whole-body glucose appearance during daytime, while net hepatic glycogenolysis contributes to whole-body glucose production only during the night.

3. Regulation of Hepatic Glycogen Turnover by Nutritional State

Studies in animals have provided evidence that hepatic glycogen phosphorylase may be active during periods of glycogen synthesis (Newman and Armstrong, 1978; van de Werve and Jeanrenaud, 1987). Simultaneous active glycogenolysis and synthesis is termed glycogen turnover or glycogen cycling (Villar-Palasi and Larner, 1960). Some authors reported that glycogen cycling is negligible in rat (Katz *et al.*, 1979) or ranges between 2 and 12 percent in healthy and diabetic humans employing [2-^{3}H,^{14}C-]galactose infusions (Wajngot *et al.*, 1991).

Combining ^{13}C NMR spectroscopy with the ^{13}C glucose pulse-^{12}C glucose chase technique demonstrated that there is a substantial amount of glycogen turnover occuring, both in rat liver (Shulman *et al.*, 1988; David *et al.*, 1990) and in human liver (Magnusson *et al.*, 1994; Roden *et al.*, 1996; Petersen *et al.*, 1998). Magnusson and colleagues (1994) studied healthy male subjects during hyperglycemia (~ 9.4 mmol/l) maintained by sequential infusion of ^{13}C-labeled and unlabeled glucose in the fed and fasted state. Fluxes through glycogen synthase were 0.43 ± 0.09 and 0.53 ± 0.12 μmol.l^{-1}.min^{-1} and through glycogen phosphorylate were 0.15 ± 0.06 and 0.30 ± 0.06 μmol.l^{-1}.min^{-1} in the fed and in the fasted states, respectively. Calculating the ratio of glucose phosphorylase flux to glycogen synthase flux yielded minimum estimates of relative glycogen turnover rate of 31 ± 8 percent in the fed state and of 57 ± 3 percent in the fasted state. Considering the linear correlation between hepatic glycogen concentration and glycogenolysis, it is conceivable that the higher liver glycogen content in the fed state may itself

stimulate glycogen breakdown by hepatic autoregulation (Clore *et al.*, 1991; Cherrington *et al.*, 1998).

4. Regulation of Hepatic Glycogen Turnover by Glucose, Insulin, and Glucagon

Hormones such as insulin and glucagon are known to affect the enzymes involved in glycogen metabolism. The rate of hepatic glycogen synthesis depends on portal vein insulin, requiring concentrations in the 130-170 pmol/l range for half-maximal stimulation of glycogen synthesis (Roden *et al.*, 1996) (Figure 4). This agrees well with the concentration for suppression of glucose production by insulin (DeFronzo *et al.*, 1983; Kolterman *et al.*, 1980; Rizza *et al.*, 1981). Although the overall flux through glycogen synthase might plateau at high insulin levels, substantial differences in net glycogen synthesis and glycogen turnover occur.

The ^{13}C-labeled glucose-^{12}C glucose chase technique was used to assess flux rates through glycogen phosphorylase in healthy humans during hypoglucagonemic (~ 30 ng/l) conditions of 1) hyperglycemic (~ 10 mmol/l)-hypoinsulinemia (~ 40 pmol/l); 2) euglycemic (~ 5 mmol/l)-hyperinsulinemia (~ 400 pmol/l); and 3) hyperglycemic (~ 10 mmol/l)-hyperinsulinemia (~ 400 pmol/l) (Petersen *et al.*, 1998) (Figure 5). Hyperglycemia *per se* inhibited net hepatic glycogenolysis, primarily through inhibition of glycogen phosphorylase flux,

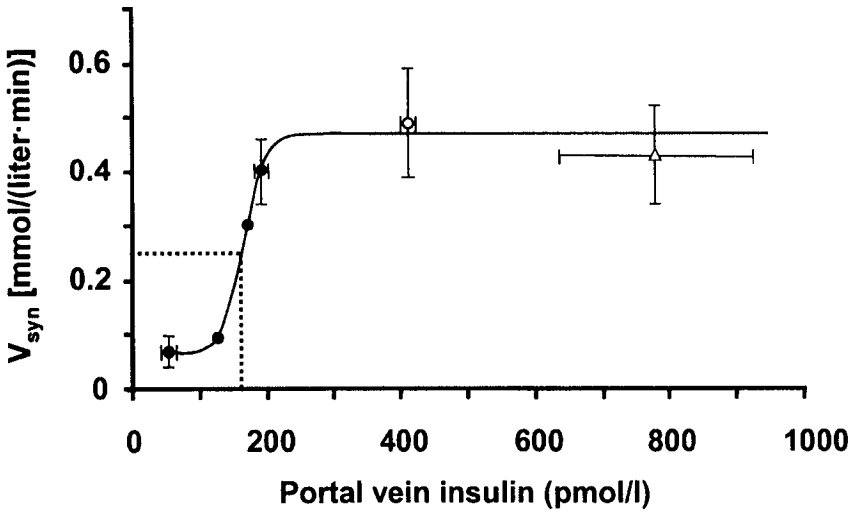
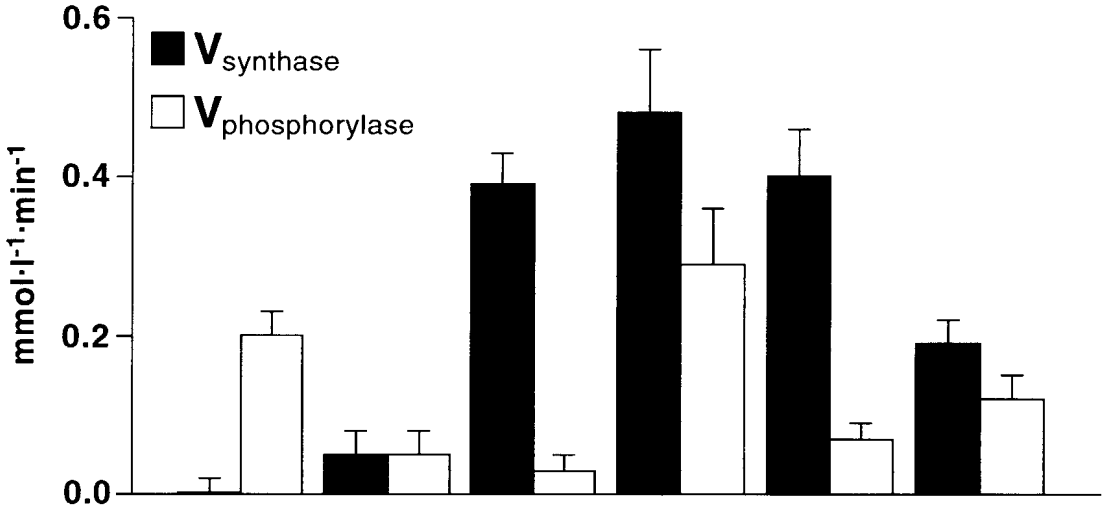


FIG. 4. Concentration dependence of mean glycogen synthetic rate at varying (portal vein) insulin concentrations under conditions of intravenous glucose infusion and suppressed glucagon secretion. [Reprinted with permission from Roden, M., Perseghin, G., Petersen, K.F., Hwang, J.H., Cline, G.W., Gerow, K., Rothman, D.L., and Shulman, G.I. *J. Clin. Invest.* **97**, 642-648, 1996.]



Glucose (mmol/l)	5	10	10	5	10	10
Insulin (pmol/l)	40	40	400	400	200	200
Glucagon (ng/l)	30	30	30	30	30	60

FIG. 5. Hepatic flux rates through glycogen synthase ($V_{synthase}$) and glycogen phosphorylase ($V_{phosphorylase}$) in healthy humans. [Data from Roden, M., Perseghin, G. Petersen, K.F., Hwang, J.H., Cline, G.W., Gerow, K., Rothman, D.L., and Shulman, G.I. *J. Clin. Invest.* **97**,642-648, 1996; Petersen, K.F., Krssak, M., Navarro, V., Chandramouli, V., Hundal, R., Schumann, W.C., Landau, B.R., and Shulman, G.I. *Am. J. Physiol.* **276**, E529-E535, 1998.]

while hyperinsulinemia *per se* inhibited net hepatic glycogenolysis, primarily through stimulation of glycogen synthase flux. When hyperglycemia was combined with hyperinsulinemia, phosphorylase flux was inhibited and glycogen synthesis was activated, resulting in the highest rates of net hepatic glycogen synthesis (Figure 5).

To determine the role of glucagon on hepatic glycogen synthesis and cycling, hyperglycemic (~ 10 mmol/l)-somatostatin clamps were performed under conditions of 1) fasting portal vein insulinemia (~ 200 pmol/l)-hypoglucagonemia (~ 30 ng/l), 2) fasting portal vein insulinemia (~ 200 pmol/l) and glucagonemia (~ 60 ng/l), and 3) fasting peripheral insulinemia (~ 50 pmol/l)-hypoglucagonemia (~ 60 ng/l) (Roden *et al.,* 1996) (Figure 5). Suppression of glucagon secretion doubled the hepatic glycogen synthase flux and decreased glycogen turnover by approximately 73 percent, so that net glycogen synthesis was markedly increased, compared with conditions of fasting plasma glucagon concentrations.

B. HEPATIC GLYCOGEN METABOLISM IN DISEASE

1. Type 1 Diabetes Mellitus (DM)

In order to examine the effect of type 1 diabetes on hepatic glycogen synthesis, patients with poorly controlled (i.e., glycosylated hemoglobin, 10.2 ± 0.3 percent) type 1 diabetes were studied during a normal-life day during which they ingested three mixed meals. Compared to healthy control subjects, these patients had similar fasting hepatic glycogen stores but, at the end of the day, they had synthesized only around 30 percent of the glycogen stored in the livers of the nondiabetic subjects studied under similar conditions (Hwang *et al.,* 1995) (Figure 6). In addition, the contribution of the gluconeogenic relative to the direct pathway of glycogen synthesis was higher in the type 1 diabetic patients over the 5 hours after breakfast (59 ± 4 percent vs. 35 ± 4 percent of control).

To examine the effect of intensified insulin treatment on hepatic glycogen metabolism, type 1 diabetic patients with moderate metabolic control (i.e., mean glycosylated hemoglobin A_{1c} of 8.8 ± 0.3 percent) were examined before and during variable insulin infusion, resulting in near-normal glycemia for 24 hours (Bischof *et al.,* 1999). After dinner, rates of net glycogen synthesis in the type 1 diabetic patients were approximately 25 percent of that in healthy volunteers and doubled during intensified insulin treatment. Nevertheless, the rate was still markedly lower, compared with the control group (0.11 ± 0.02 vs. 0.23 ± 0.04 $\mu mol.l^{-1}.min^{-1}$). During overnight fasting, net hepatic glycogenolysis was reduced by about 50 percent in the type 1 diabetic patients and increased by around 40 percent by intensified insulin treatment but was still lower than in the control group (0.14

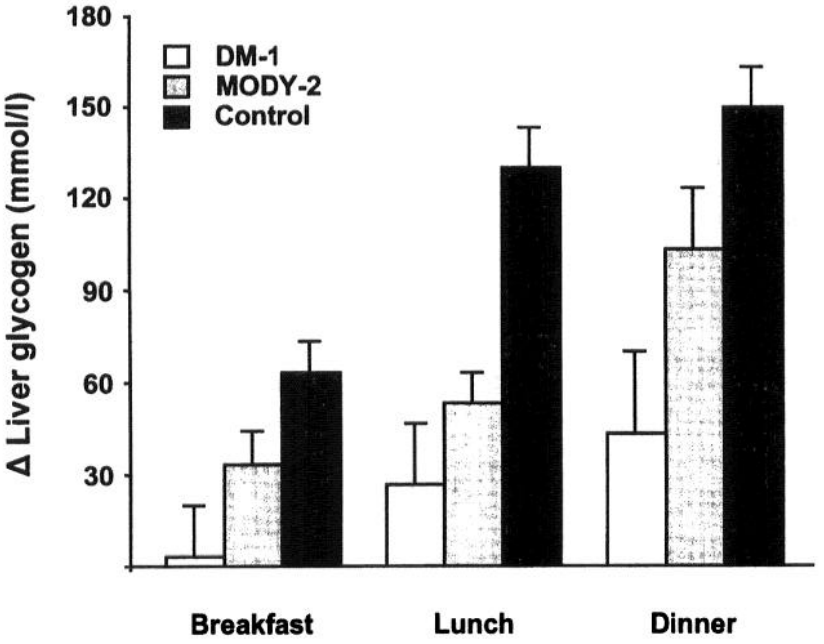


FIG. 6. Increments in hepatic glycogen concentration in type 1 diabetic (DM-1), maturity-onset diabetic (MODY-2), and healthy subjects (CONTROL) during a day in which three isocaloric mixed meals were ingested 5 hours apart. [Reprinted with permission from Hwang, J.H., Perseghin, G., Rothman, D.L., Cline, G.W., Magnusson, I., Petersen, K.F., and Shulman, G.I. *J. Clin. Invest.* **95,** 783-787, 1995; and Velho, G., Petersen, K.F., Perseghin, G., Hwang, J.H., Rothman, D.L., Pueyo, M.E., Cline, G.W., Froguel, P., and Shulman, G.I. *J. Clin. Invest.* **98,** 1755-1761, 1996.]

$\pm$ 0.01 vs. 0.19 $\pm$ 0.01 $\mu mol.l^{-1}.min^{-1}$). Thus, short-term improvement of metabolic control resulting in near-normoglycemia and peripheral hyperinsulinemia was not sufficient to completely reverse the abnormalities of hepatic glycogen metabolism in type 1 diabetes.

On the other hand, in the presence of both hyperglycemia and hyperinsulinemia, rates of hepatic glycogen synthesis did not differ between poorly controlled (mean glycosylated hemoglobin, 14.1 $\pm$ 1.7 percent) type 1 diabetic and nondiabetic subjects (0.49 $\pm$ 0.10 vs. 0.46 $\pm$ 0.07 $\mu mol.l^{-1}.min^{-1}$) (Cline *et al.*, 1994). These data suggest that glycogen synthesis can be normalized under high-glucose, high-insulin, but low-glucagon conditions in type 1 diabetic patients. However, the percent contribution of the direct pathway to glycogen synthesis

remained lower in type 1 diabetic than in nondiabetic subjects, despite the presence of hyperinsulinemia (~ 35 vs. 60 percent). This agrees with the results obtained after meal ingestion (Hwang *et al.*, 1995), reflecting higher gluconeogenic rates that are not acutely normalized by hyperinsulinemia (Cline *et al.*, 1994).

2. Type 2 DM

It is well established that fasting hyperglycemia in patients with type 2 diabetes is strongly related to increased rates of endogenous glucose production (Fery, 1994; Bogardus *et al.*, 1984; DeFronzo, 1997). These increased rates of glucose production could be secondary to increased rates of gluconeogenesis, hepatic glycogenolysis, or both. In order to address this question, rates of net hepatic glycogenolysis were measured in patients with poorly controlled type 2 diabetes (mean hemoglobin A_{1c}, 12 ± 1 percent) and gluconeogenesis was calculated as the difference between rates of glycogenolysis and whole-body glucose production (Magnusson *et al.*, 1992) (Figure 7). Despite pair feeding, liver glycogen concentration was lower in type 2 diabetic than in healthy control subjects at 4 hours after a meal (131 ± 20 vs. 282 ± 60 mmol/l liver), consistent with a defect in hepatic glycogen synthesis in type 2 diabetes (Magnusson *et al.*, 1992). The postprandial rise in the rate of whole-body glucose production (around 25 percent), estimated by using [6-^{3}H]glucose, was accompanied by decreased rates of net hepatic glycogenolysis (around 50 percent). Thus, increased gluconeogenesis could completely account for the higher rates of glucose production obtained in patients with poorly controlled type 2 diabetes.

The effect of the oral hypoglycemic drug, metformin, which decreases fasting plasma glucose concentrations by reducing hepatic glucose production (Cusi and DeFronzo, 1997; Inzucchi *et al.*, 1998), was examined in poorly controlled (i.e., fasting plasma glucose, ~ 15.5 mmol/l) type 2 diabetic patients by employing two independent methods: 1) ^{13}C NMR spectroscopy of the liver, combined with determination of glucose production with [6,6-^{2}H]glucose, gives the rate of net hepatic glycogenolysis to glucose production; and 2) the ratio of ^{2}H enrichments in carbon 5 over carbon 2 in blood glucose upon administration of ^2H_2O gives the contribution of gluconeogenesis to glucose production (Landau *et al.*, 1995,1996). Rates of glucose production and gluconeogenesis were two- and three-fold higher in diabetic than in healthy subjects (Hundal *et al.*, 2000). Treatment with metformin for 3 months reduced fasting plasma glucose concentration by approximately 30 percent, glucose production by approximately 24 percent, and gluconeogenesis by approximately 36 percent. Employing the ^2H_2O method, gluconeogenesis was two-fold higher in the diabetic patients and was reduced by

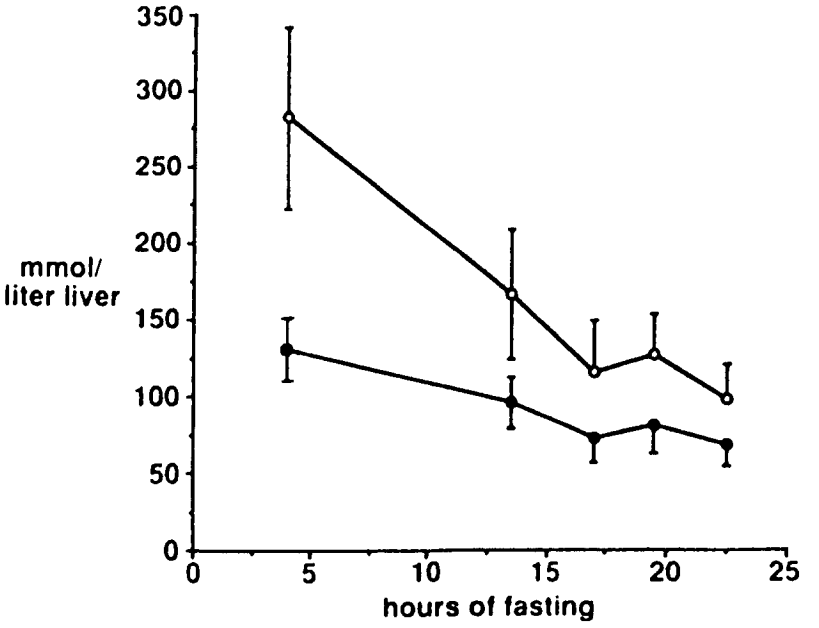


FIG. 7. Time course of mean liver glycogen concentration in type 2 diabetic and healthy subjects. [Reprinted with permission from Magnusson, I., Rothman, D.L., Katz, L.D., and Shulman, G.I. *J. Clin. Invest.* **90,** 1323-1327, 1992.]

around 33 percent by metformin treatment. Net hepatic glycogenolysis (by ^{13}C NMR) was lower before (0.11 ± 0.02 mmol.m^{-2}.min^{-1}) but almost normalized after metformin therapy (0.15 ± 0.02 mmol.m^{-2}.min^{-1} vs. control of 0.18 ± 0.03 mmol.m^{-2}.min^{-1}). Since ^{13}C NMR spectroscopy measures net hepatic glycogenolysis, while the $^{2}H_2O$ method indirectly measures total glycogenolysis (glucose production minus gluconeogenesis), the difference between total and net glycogenolysis can be used to assess hepatic glycogen cycling. Glycogen cycling was negligible in healthy subjects but contributed as much as 25 percent of glucose production in type 2 diabetic patients. The reduction of gluconeogenesis by metformin also may be due in part to the reduction of plasma free fatty acids, which have been shown to increase gluconeogenesis (Chen *et al.*, 1999; Roden *et al.*, 2000; Stingl *et al.*, in press).

3. Maturity Onset Diabetes of Young 2 (MODY2)

Studies in a familial subgroup of diabetes, MODY 2, have shed some light on the potential intrahepatic defects occurring in type 2 DM. MODY 2 subjects present with moderate (postprandial) hyperglycemia due to autosomal dominant mutations in the coding regions of the hepatic and pancreatic isoforms of the glucokinase gene (Stoffel *et al.,* 1992; Froguel *et al.,* 1993). During a study employing three mixed meals over 1 day, the net increase in liver glycogen was 30-60 percent lower in MODY 2 than in control subjects. At the end of the day, glucokinase-deficient subjects had only about 70 percent of the increment in glycogen observed in control subjects (Figure 6) (Velho *et al.,* 1996). In addition, the relative contribution of the gluconeogenic pathway to glycogen synthesis was higher in these subjects during the 4 hours after breakfast (50 ± 2 percent vs. 34 ± 5 percent of control). Since relative hypoinsulinemia was present in the MODY 2 patients only during the first 2 hours following breakfast, the decreased insulin/glucagon ratio cannot entirely explain the reduction of liver glycogen synthesis following lunch and dinner and suggests that decreased activity of hepatic glucokinase is responsible for the impaired glycogen storage and postprandial hyperglycemia during these periods. From these results, one might speculate that the similar defects of liver glucose metabolism observed in patients with poorly controlled type 1 and type 2 DM might be related at least in part to an acquired abnormality of hepatic glucokinase.

4. Liver Cirrhosis

Hepatic glucose metabolism is generally thought to be altered in liver cirrhosis, although rates of glucose production were found to be decreased only in some (Owen *et al.,* 1981; Merli *et al.,* 1986) but not all studies (Shmueli *et al.,* 1993; Petrides *et al.,* 1991). To examine hepatic glycogen metabolism, Petersen and coworkers (1999) studied patients with biopsy-confirmed liver cirrhosis by combining ^{13}C NMR spectroscopy with [6,6-^{2}H]glucose infusion and the $^{2}H_2O$ method described above. Despite comparable rates of glucose production, net hepatic glycogenolysis was about 3.5-fold lower in cirrhotic patients (1.1 ± 0.4 $\mu mol.kg^{-1}.min^{-1}$ vs 3.9 ± 0.9 $\mu mol.kg^{-1}.min^{-1}$) and the relative contribution of gluconeogenesis was markedly increased. Using the $^{2}H_2O$ method, the contribution of gluconeogenesis to glucose production was also found to be markedly higher in the cirrhotic patients compared to the healthy subjects (68 ± 3 percent vs. 54 ± 2 percent). From these data, rates of total glycogenolysis can be calculated to be approximately 3.2 $\mu mol.kg^{-1}.min^{-1}$ and 4.6 $\mu mol.kg^{-1}.min^{-1}$, respectively. Glycogen cycling, as assessed from the difference between total and net glyco-

genolysis, contributed to about 23 percent and 7 percent of glucose production in cirrhotic and healthy subjects, respectively. Increased glycogen cycling in liver cirrhosis may result from the approximately 2.5-fold higher plasma concentrations of insulin and glucagon, both of which stimulate glycogen turnover (Magnusson *et al.*, 1994; Petersen *et al.*, 1998; Roden *et al.*, 1996). These patients also had increased plasma concentrations of free fatty acids, which stimulate glycogen cycling by increasing gluconeogenesis (Chen *et al.*, 1999; Roden *et al.*, 2000) and decreasing net hepatic glycogenolysis (Stingl *et al.*, in press). Finally, the lower insulin-like growth factor-I (IGF-I) concentrations may favor skeletal muscle proteolysis with subsequent stimulation of gluconeogenesis by amino acids in patients with liver cirrhosis.

5. Glycogen Storage Diseases

^{13}C NMR spectroscopy also may be useful to identify patients with glycogen storage disease. Elevated hepatic glycogen concentrations were reported in one patient with type IIIA glycogenosis (Beckmann *et al.*, 1990), which is characterized by inactivation of the amylo-1,6-glucosidase debrancher enzyme, and in one patient with type IA glycogenosis, which is due to deficiency of glucose-6-phosphatase (Roser *et al.*, 1996).

IV. Summary and Conclusions

Application of NMR spectroscopy offers the unique ability to noninvasively and continuously monitor intracellular substrates in humans. With this method, the time course of hepatic glycogen concentrations can be followed both in the fasted and in the postprandial state *in vivo.* Using this technique, substrate fluxes through metabolic pathways such as glycogen synthase and glycogen phosphorylase can be examined. Moreover, NMR spectroscopy has made it feasible to detect defects of hepatic glycogen accumulation in poorly controlled type 1, type 2, and MODY-2 diabetic patients. NMR spectroscopy identified increased gluconeogenesis as the major cause of elevated glucose production in patients with poorly controlled type 2 diabetes. These studies demonstrate that NMR spectroscopy can be applied effectively in humans and that it has provided new insights into the pathogenesis of type 1, type 2, and MODY-2 diabetes.

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