Contribution of Net Hepatic Glycogen Synthesis to Disposal of an Oral Glucose Load in Humans

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The contribution of hepatic glycogen synthesis to whole body glucose disposal after an oral glucose load was examined using ^{13}C nuclear magnetic resonance (NMR) spectroscopy to measure liver glycogen content in healthy, volunteers after an overnight fast. In group 1 (n = 14), hepatic glycogen synthesis was measured using ^{13}C-NMR spectroscopy for 240 minutes after ingestion of 98 ± 1 g glucose. Liver volumes were measured using magnetic resonance imaging (MRI). To assess the direct (glucose → glucose-6-P → glucose-1-P → uridine diphosphate (UDP)-glucose → glycogen) and indirect (3-carbon units → → glycogen) pathways of liver glycogen synthesis, group 2 (n = 6) was studied with an identical glucose load enriched with [1-^{13}C]glucose along with acetaminophen to noninvasively assess the ^{13}C enrichment in hepatic UDP-glucose. The fasting hepatic glycogen content was 305 ± 17 mmol/L liver, and the liver volume was 1.46 ± 0.07 L. For the initial 180 minutes after ingestion of glucose, hepatic glycogen concentrations increased linearly (r = .94, P = .0006) achieving a maximum concentration of 390 ± 7 mmol/L liver and then remained constant until the end of the study. The mean maximum rate of net hepatic glycogen synthesis was 0.48 ± 0.07 mmol/L liver-minute. Total liver glycogen synthesis could account for 16.7 ± 3.8 g (17% ± 4%) of the glucose ingested, and of this, 10.5 ± 2.4 g (63% ± 7%) was synthesized by the direct pathway. In conclusion, after ingestion of 98 g of glucose: (1) 16.7 ± 3.8 g (17% ± 4%) glucose was stored in the liver as glycogen, and (2) 63% ± 7% (10.5 ± 2.4 g) of this glycogen was formed via the direct pathway.

PREVIOUS STUDIES IN humans have shown that liver glycogen synthesis accounts for approximately 10% to 15% of intravenously infused glucose under hyperglycemic-hyperinsulinemic conditions.[1] However, little is known about the role of liver glycogen synthesis for glucose disposal under the more physiologic conditions of oral glucose ingestion. This is a critical issue given the importance of the portal-arterial gradient in determining glucose uptake by the liver.[2-5] After a carbohydrate meal, liver glycogen is synthesized by both the direct [glucose → glucose-6-phosphate → glucose-1-phosphate → uridine diphosphate (UDP)-glucose → glycogen] and indirect (via 3-carbon compounds → glycogen) pathways, and the indirect pathway contributes approximately 40% to the total amount of liver glycogen synthesized after a glucose load in all species studied so far, including man.[6-11] It has been suggested that glucokinase, which generates glucose-6-phosphate for glycogen synthesis, is a rate-limiting factor for the synthesis of liver glycogen by the direct pathway.[11,12] However, studies by Moore et al[13] in awake dogs during intraduodenal glucose administration have shown that net glucose uptake by the liver can account for all of the liver glycogen synthesized by both the direct and indirect pathways. Because net glucose uptake by the liver might be expected to reflect a minimal flux through glucokinase, these data suggest that glucokinase is not a rate-determinaing factor for the direct pathway of hepatic glycogen synthesis. Whether the same situation occurs in humans is unknown.

To examine how much of an oral glucose load is net deposited in the liver as glycogen in humans, we used ^{13}C nuclear magnetic resonance (NMR) spectroscopy and magnetic resonance imaging (MRI) techniques to directly measure liver glycogen synthesis in humans after ingestion of 98 g of glucose. The relative contributions of the direct and indirect pathways of glycogen synthesis were estimated using [1-^{13}C]glucose in conjunction with acetaminophen as a noninvasive probe of the hepatic UDP-glucose pool.[14,15]

MATERIALS AND METHODS

Methods

Subjects. Twenty, lean healthy, nonsmoking volunteers (12 men and 8 women; age, 26 ± 2 years; weight, 74 ± 4 kg), ingested a weight-maintaining diet containing 250 to 300 g carbohydrates for 3 days before the study while avoiding any type of strenuous physical activity. Group 1 (n = 14) underwent ^{13}C NMR spectroscopic measurements of liver glycogen concentrations before and after an oral glucose load. Group 2 (n = 6) were given 1.5 g acetaminophen with the oral glucose load to determine the percent contribution of the direct and indirect pathways of hepatic glycogen synthesis. All studies were begun at 7 AM after an overnight fast of 10 to 11 hours with insertion of an intravenous catheter in an antecubital vein for blood collection. The experimental protocol was approved by the Human Investigation Committee of Yale University School of Medicine, and informed consent was obtained from each participant.

Group 1. The subjects were brought to the Yale University Magnetic Resonance Center in a wheelchair, placed in a NMR spectrometer (1 m bore, 2.1T; Bruker Biospec Spectrometer, Billerica, MA) and a $^{1}H/^{13}C$ concentric surface coil (embedded in a 6 mm thick Lucite plate) was placed over the lateral aspect of the abdomen after percussion of the liver borders. The position of the coil was verified by a multislice gradient-echo image with a 2-cm formate sphere attached to the center

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of the coil as a calibration marker. Localized ^{13}C-NMR liver spectra were obtained using a multislice gradient echo image as described previously.[16] Fasting hepatic glycogen concentrations were measured with ^{13}C-NMR spectroscopy during a 60-minute baseline period, where after an oral dose of 98 ± 1 g of glucose was given in a 150-mL Glucola solution (Curtin Matheson Scientific, Houston, TX) while the subjects were lying inside the spectrometer. Allowing time for absorption, hepatic glycogen concentrations were measured continuously from time 30 to 240 minutes. Blood was collected for plasma glucose and insulin concentrations every 15 minutes.

Group 2. After collection of fasting blood samples, 1.5 g acetaminophen was administered orally along with regular drinking water followed 30 minutes later by 98 g of glucose (88 g unlabelled glucose to which 10 g of [1-^{13}C]glucose was added in a total volume of 150 mL [Glucola; Curtin Matheson Scientific, Houston, TX]). Blood was collected for plasma glucose and insulin concentrations every 15 minutes and for ^{13}C glucose and ^{13}C glucuronide enrichments every 30 minutes.

Liver volume. Liver volume was measured at 7 AM before the glucose ingestion, using MRI with 3-dimensional reconstruction in a 1.5 T Magnetic Resonance Imager (Signa; General Electric Co, Milwaukee, WI) as described previously.[17]

Plasma analyses. Plasma glucose concentrations were measured by the glucose oxidase method (Glucose Analyzer II; Beckman Instruments, Fullerton, CA). Plasma immunoreactive insulin concentrations were measured using a double antibody radioimmunoassay kit (Diagnostic Sys. Labs, Webster, TX). Plasma glucose and acetaminophen-glucuronide were derivatized for determination of ^{13}C atom percent enrichments (APE) by gas chromatography-mass sprectometry (GC-MS). Plasma glucose was derivatized as the pentaacetate after $Ba(OH)_2/ZnSO_4$ deproteinization and semipurification by anion/cation exchange chromatography (AG1-X8; AG50W-X8, Bio-Rad Lab, Richmond, CA), as described previously.[18] Plasma acetaminophen-glucuronide was derivatized by a modification of that used for amino acids.[1] Plasma was deproteinized with $Ba(OH)_2/ZnSO_4$, the supernatant freeze-dried, and the glucuronide moiety was derivatized as the n-butyl ester, triacetate.

GC-MS analysis. GC-MS analysis was performed with a Hewlett-Packard (Palo Alto, CA) 5890 gas chromatograph (HP-1 capillary column, 12 m × 0.2 mm × 0.33 μm film thickness) interfaced to a Hewlett-Packard 5971A Mass Selective Detector operating in the positive chemical ionization mode (with methane as reagent gas) for acetaminophen-glucuronide enrichment as described earlier.[19]

Calculations

The total hepatic glycogen content was calculated by multiplying the molar concentration by the MRI determined liver volume, and the rate of net hepatic glycogen synthesis was estimated from the initial increase in hepatic glycogen content (assuming a molecular weight of 180 g/mol) over the first 180 minutes.

The fraction of UDP-glucose formed from the direct pathway of glycogen synthesis was determined from the ^{13}C enrichment in carbon 1 (C1) and carbon 6 (C6) of plasma glucose and acetaminophen-glucuronide and calculated as: [^{13}C-APE of plasma glucuronide (C1-C6)]/[^{13}C APE of plasma glucose (C1-C6)]; where APE is atom percent enrichment. The average plasma ^{13}C-APE of acetaminophen-glucuronide was calculated for 3 time intervals: 60 to 120 minutes, 120 to 180 minutes, and 180 to 240 minutes.[19] All data are expressed as mean ± SEM.

RESULTS

Plasma glucose concentrations increased from 5.0 ± 0.1 mmol/L fasting to 7.5 ± 0.3 mmol/L 30 minutes after the glucose ingestion and then declined reaching fasting concentrations of 4.8 ± 0.3 mmol/L approximately 180 minutes after the glucose intake (Fig 1A). The plasma insulin concentrations increased after the glucose load from 47 ± 56 pmol/L at time 0 minutes to a maximum of 429 ± 60 pmol/L by 75 minutes and returned to baseline concentrations (57 ± 5 pmol/L) by 240 minutes (Fig 1B).

Fasting hepatic glycogen concentrations were 305 ± 17 mmol/L, liver and the liver volume was 1.464 ± 0.073 liter (n = 14). The time course for liver glycogen concentrations is shown in Fig 1C. After ingestion of the glucose load, liver glycogen concentrations increased in a linear fashion from 305 ± 17 mmol/L liver to 390 ± 7 mmol/L liver at 180 minutes (r = .94, P = .0006). During this initial phase, the mean rate of net hepatic glycogen synthesis was 0.48 ± 0.07 mmol/(L liver-min). Total liver glycogen synthesis as calculated from the increase in glycogen content over the first 180 minutes was 16.7 ± 3.8 g, which represents 17% ± 4% of the 98 ± 1 g glucose administered. During the remainder of the study period (180 to 240 minutes), the liver glycogen concentrations remained constant at 390 ± 7 mmol/L liver.

The relative contribution of the direct pathway of hepatic glycogen synthesis calculated from the meal ^{13}C glucose enrichment divided by the ^{13}C enrichment of the plasma acetaminophen-glucuronide was 62% ± 6% from 60 to 120 minutes, 64% ± 10% from 120 to 180 minutes, and 64% ± 17% from 180 to 240 minutes.

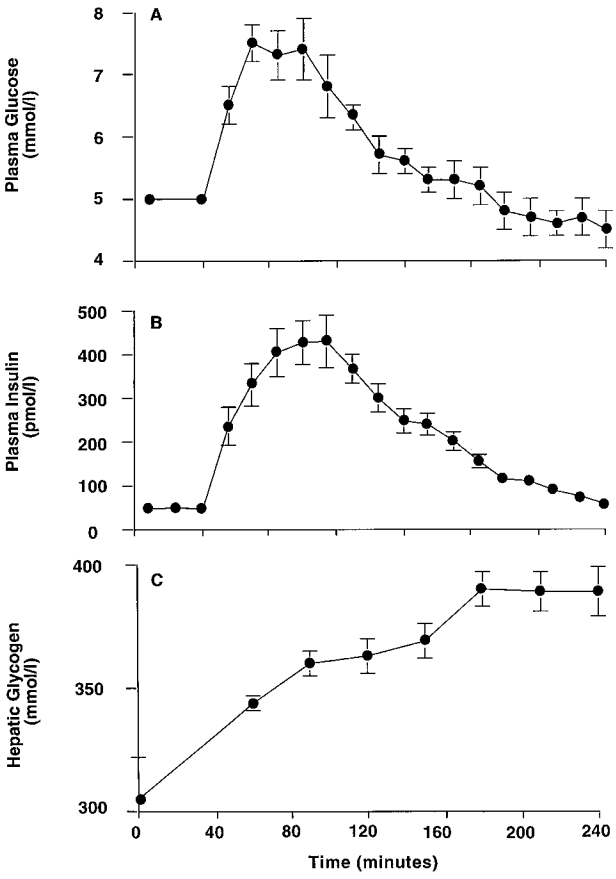

Fig 1. Time course of concentrations of plasma glucose (A), plasma insulin (B), and hepatic glycogen (C) after oral administration of 98 g glucose.

With the percent direct pathway accounting for on average 63% ± 7%, 10.5 ± 2.4 g glycogen was synthesized via the direct pathway, corresponding to 11% ± 2% of the oral glucose load.

DISCUSSION

After an oral glucose load, the liver plays a critical role in maintaining glucose homeostasis, both by suppressing glucose production and switching from net glucose production to net glucose uptake. Furthermore, this latter process is known to be enhanced by oral versus intravenous delivery of glucose.[20-23] Surprisingly little is known about the fate of an oral glucose load in humans. To address this question, we measured net hepatic glycogen synthesis after a standard 98-g glucose load and found that approximately 17 g were deposited as liver glycogen, which accounted for approximately 17% of the glucose load.

Only 1 previous study in humans has measured hepatic glycogen synthesis directly after glucose ingestion.[24] In this study, Beckmann et al[24] measured hepatic glycogen concentrations (mmol/L) with ^{13}C-NMR spectroscopy during the initial 2 hours after ingestion of variable amounts of glucose (50 to 250 g) and found that the amount of the ingested glucose stored as liver glycogen increased with the size of the glucose load so approximately 24% to 32% of the ingested glucose was stored as liver glycogen. This is more than our estimate of 17%. However, in this study, the prestudy diets of the subjects were not standardized so the initial liver glycogen concentrations were low ranging from 160 to 236 mmol/L, and the number of subjects in each of the 3 groups was small (2 to 5 subjects).

Radziuk[21] addressed the question of the fate of an oral glucose load in humans by calculating initial splanchnic glucose uptake after a small (45 g) and a large (96 g) glucose meal using a multiradiolabeled tracer ([3-^{3}H]glucose, [1-^{14}C]glucose, and [2-^{3}H]glucose) approach and a bolus injection of glucagon to wash out newly synthesized glycogen. He found that splanchnic glucose uptake accounts for 8% of the ingested glucose, which was about half of our estimate. However, these studies were indirect, limited to a few study subjects and relied on several assumptions of pool sizes, reaction equilibriums, and the effects of glucagon being selective on newly formed glycogen (and having no effects on gluconeogenesis).[21]

The second question we addressed, how much glycogen was synthesized by the direct and the indirect pathways after the oral glucose intake, was assessed using acetaminophen to noninvasively sample the hepatic UDP-glucose pool, which contributes the glucosyl units to glycogen formation.[15,25] By isolating acetaminophen-glucuronide from plasma, the atom percent ^{13}C enrichment of the glucosyl units of the newly formed glycogen can be determined. Using this approach, we estimated that the contribution of the direct pathway of hepatic glycogen synthesis to the total amount of glycogen synthesized after the glucose meal was 63% ± 7%. Thus, of the 16.7 ± 3.8 g net glycogen synthesized, 10.5 ± 2.4 g of glycogen was synthesized via the direct pathway and 6.2 ± 1.4 g via the indirect pathway. These results are in good agreement with our previous results,[26] as well as a recent study by Hellerstein et al[27] who used the acetaminophen-glucuronate probe technique and found that the contribution of the direct pathway during a 9-hour intravenous glucose infusion was 62% to 64%.

The relative contribution of the direct pathway to hepatic glycogen synthesis was examined by Radziuk[22,23] using multiradiolabeled tracer ([1-^{14}C]glucose, [3-^{3}H]glucose, [6-^{3}H]glucose, [U-^{14}C]lactate, [^{14}C]bicarbonate) infusions in conjunction with a glucagon infusion to flush out hepatic glycogen, to indirectly assess glucose absorption, hepatic glycogen synthesis, and the pathways of glycogen synthesis. Although the approach is indirect and has many assumptions as discussed earlier, he found that approximately 8 to 10 g of the administered glucose entered hepatic glycogen via the direct pathway, which is similar to our estimate of 10.5 g.

It has been argued that glucokinase is the determining factor for the amount of hepatic glycogen synthesized by the direct pathway.[11] However, studies by Moore et al[13] in dogs have challenged this concept by showing that the liver is capable of taking up enough glucose to account for all of the glycogen synthesized by both the direct and indirect pathways. In human volunteers, such direct measurements of net hepatic uptake are untenable, but 3 studies have used arterial-hepatic venous difference techniques to measure splanchnic glucose output after 92-g glucose meals in normal humans and arrived at very similar results regarding net splanchnic glucose uptake.[5,28,29] In these 3 studies, over the first 180 minutes after ingestion of the glucose, the average net splanchnic glucose output for the 3 studies was 56 ± 3 g. Thus approximately 36 g glucose (39% of the glucose meal of 92 g) were retained in the splanchnic bed during the initial 3 hours after glucose ingestion. In our studies, splanchnic glucose uptake would have been 38 g (39% of the 98 g administered). Assuming nonhepatic splanchnic (gut) glucose metabolism accounts for approximately 8% of the splanchnic glucose uptake (8% of 38 g, ≃ 3 g), it can be estimated that net hepatic glucose uptake was approximately 35 g glucose equivalents. This is enough glucose to account for all of the hepatic glycogen synthesized (16.7 g) and is consistent with the previous results in awake dogs.[13] Because virtually all of the glucose taken up by the liver is metabolized by glucokinase to glucose-6-phosphate, these data suggest that glucokinase is not rate-limiting for the direct pathway of hepatic glycogen synthesis in humans and suggests that there is a substantial amount of substrate cycling between glycolysis and gluconeogenesis in the liver under these conditions, which is supported by previous studies.[13,30,31]

In summary, after a 98-g glucose meal: (1) net liver glycogen synthesis accounts for 16.7 ± 3.8 g (17% ± 4%) the glucose ingested by humans, and (2) the direct pathway of glycogen synthesis contributes 63% ± 7% (10.5 ± 2.4 g) and the indirect pathway contributes 37% (6.2 ± 1.4 g) to net hepatic glycogen synthesis.

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