Stimulating Effects of Low-Dose Fructose on Insulin-Stimulated Hepatic Glycogen Synthesis in Humans

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Fructose has been shown to have a catalytic effect on glucokinase activity in vitro; however, its effects on hepatic glycogen metabolism in humans is unknown. To address this question, we used ^{13}C nuclear magnetic resonance (NMR) spectroscopy to noninvasively assess rates of hepatic glycogen synthesis and glycogenolysis under euglycemic (~5 mmol/l) hyperinsulinemic conditions (~400 pmol/l) with and without a low-dose infusion of fructose (~3.5 μmol · kg^{-1} · min^{-1}). Six healthy overnight-fasted subjects were infused for 4 h with somatostatin (0.1 μg · kg^{-1} · min^{-1}) and insulin (240 pmol · m^{-2} · min^{-1}). During the initial 120 min, [1-^{13}C]glucose was infused to assess glycogen synthase flux followed by an ~120-min infusion of unlabeled glucose to assess rates of glycogen phosphorylase flux. Acetaminophen was given to assess the percent contribution of the direct and indirect (gluconeogenic) pathways of glycogen synthesis by the ^{13}C enrichment of plasma UDP-glucuronide and C-1 of glucose. In the control studies, the flux through glycogen synthase and glycogen phosphorylase was 0.31 ± 0.06 and 0.17 ± 0.04 mmol/l per min, respectively, and the rate of net hepatic glycogen synthesis was 0.14 ± 0.05 mmol/l per min. In the fructose studies, the glycogen synthase flux increased 2.5-fold to 0.79 ± 0.16 mmol/l per min (*P* = 0.018 vs. control), whereas glycogen phosphorylase flux remained unchanged (0.24 ± 0.06; *P* = 0.16 vs. control). The infusion of fructose resulted in a threefold increase in rates of net hepatic glycogen synthesis (0.54 ± 0.12 mmol/l per min; *P* = 0.008 vs. control) without affecting the pathways of hepatic glycogen synthesis (direct pathway ~60% in both groups). We conclude that during euglycemic hyperinsulinemia, a low-dose fructose infusion causes a threefold increase in net hepatic glycogen synthesis exclusively through stimulation of glycogen synthase flux. Because net hepatic glycogen synthesis has been shown to be diminished in patients with poorly controlled type 1 and type 2 diabetes, stimulation of hepatic glycogen synthesis by this mechanism may be of potential therapeutic value. *Diabetes* 50:1263–1268, 2001

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APE, atom percent enrichment; GC-MS, gas chromatography–mass spectrometry; GCRC, General Clinical Research Center; NMR, nuclear magnetic resonance.

Glucokinase activity is acutely regulated by interaction with a regulatory protein (1) that binds to fructose-6-phosphate (P) and allosterically inhibits glucokinase by lowering the enzyme's affinity for glucose. In contrast, when the regulatory protein binds to fructose-1-P, glucokinase dissociates and becomes fully active (1). Small amounts of fructose have been shown to acutely elevate fructose-1-P concentrations in the hepatocytes and may cause release of glucokinase from the regulatory protein (2–4). Shiota et al. (5) showed that in dogs under conditions of hyperglycemia and hyperinsulinemia, intraportal low-dose infusions of fructose triple the amount of hepatic glucose uptake and increase the direct pathway of glycogen synthesis (glucose → glucose-6-P → glucose-1-P → UDP-glucose → glycogen) to account for ~90% of glycogen synthesis. Whether small amounts of fructose have a similar effect on hepatic glycogen metabolism in humans remains unknown.

To address this question, we applied a ^{13}C nuclear magnetic resonance (NMR) spectroscopic technique to assess net hepatic glycogen synthesis as well as flux through glycogen synthase and phosphorylase in humans (6–8). Using this technique, we examined the effects of a small dose of fructose during a glucose-insulin clamp on rates of hepatic glycogen synthesis by monitoring the rate of [1-^{13}C]glucose incorporation into the C1 position of the glycosyl moieties of hepatic glycogen. Correction for the relative contribution of the indirect pathway was performed by monitoring the relative ^{13}C enrichments in plasma acetaminophen-glucuronide and glucose after an oral dose of acetaminophen (9,10). Flux through glycogen phosphorylase was assessed by replacing the [1-^{13}C]glucose infusate with an unlabeled glucose infusate for the last 2 h of the glucose-insulin clamp and monitoring the amount of ^{13}C-labeled glycogen replaced by unlabeled glycogen (7).

RESEARCH DESIGN AND METHODS

Six healthy sedentary volunteers (three men and three women, aged 26 ± 2 years, body weight 63 ± 2 kg, BMI 22 ± 1 kg/m^2, glycosylated hemoglobin 5.6 ± 0.2% [normal range 4–8%]) were studied in two separate 4-h euglycemic-hyperinsulinemic clamp studies with and without a concomitant low-dose infusion of fructose. The experimental protocol was reviewed and approved by the Yale University Human Investigation Committee, and informed written consent was obtained from all subjects. An isocaloric diet consisting of 60% carbohydrate, 20% protein, and 20% fat (33 kcal/kg body wt daily), prepared by the metabolic kitchen of the Yale–New Haven Hospital General Clinical

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Research Center (GCRC), was given for 3 days before each study. During these days, the subjects were instructed to limit their activity to normal walking. On the day before the study, the subjects were admitted to the GCRC at 6:00 P.M., given dinner (690 ± 22 kcal; 60% carbohydrate, 20% fat, 20% protein), and then fasted overnight. The studies were performed in random order and spaced 6–12 weeks apart. To minimize changes in ovarian hormonal effects on glucose metabolism, female subjects were studied during the follicular phase (day 0–12) of the menstrual cycle.

Experimental procedures. On the morning of the study, a Teflon catheter was placed in an antecubital vein in the left arm for infusion, and another catheter was placed in an antecubital vein in the right arm for blood withdrawal. The subjects were brought to the Yale Magnetic Resonance Center in a wheelchair and positioned in the 2.1-Tesla NMR spectrometer. At 5 min before the start of the clamp, an infusion of somatostatin was begun at a rate of 0.1 $\mu g \cdot kg^{-1}$ body wt $\cdot$ min^{-1} and continued throughout the study to inhibit endogenous insulin and glucagon secretion (11). At time 0 min, a euglycemic-hyperinsulinemic clamp was begun by raising plasma insulin levels to ~400 pmol/l with a primed constant infusion of insulin at a rate of 240 pmol $\cdot$ m^{-2} $\cdot$ min^{-1} insulin (insulin lispro; Eli Lilly, Indianapolis, IN) and maintaining plasma glucose concentrations at ~5 mmol/l by a variable infusion of [1-^{13}C]glucose (1.11 mol/l, ~20% ^{13}C-enriched) (7). At the same time, an infusion of fructose was initiated (3.5 ± 0.2 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) and continued to the end of the study at 240 min. At time 120 min, the [1-^{13}C]glucose infusate was switched to an unenriched glucose infusate (1.11 mol/l), which was used until the end of the experiment. In the control studies, a similar clamp procedure was followed, but no fructose was given. An oral dose of acetaminophen (1,300 mg) was given at the start of the [1-^{13}C]glucose infusion to estimate the dilution of [1-^{13}C]glucose from the indirect pathway in the hepatic UDP-glucose pool (9). Plasma glucose concentrations were measured every 5 min during the clamp, and blood samples were taken every 15 min for determination of plasma insulin, glucagon, and lactate and fatty acid concentrations, as well as for gas chromatography–mass spectrometry (GC-MS) analysis of ^{13}C isotopic enrichment in plasma glucose and acetaminophen glucuronide. Mean glucose infusion rates during the clamp studies were calculated in 20-min intervals starting at 60 min and corrected for change in plasma glucose concentrations.

In vivo ^{13}C NMR spectroscopic techniques. To assess rates of hepatic glycogen synthesis and breakdown, ^{13}C NMR spectra were acquired from 60 to 155 min and from 185 to 240 min. The measurements were limited to these time periods to minimize the discomfort of the subjects from lying motionless inside the 2.1-Tesla 1-m bore spectrometer (Biospec I; Bruker Instruments, Billerica, MA). The ^{13}C NMR signals were obtained with a 9-cm circular ^{13}C observation coil and a 12 × 14–cm coplanar butterfly ^{1}H-decoupler coil secured rigidly over the lateral aspect of the abdomen of the supine subject, and the C1 glycogen resonance (100.6 ppm) was quantitated by integration and comparison with a solution glycogen as previously described (12).

Analytical procedures. Plasma glucose concentrations were measured by the glucose oxidase method (Glucose Analyzer II; Beckman Instruments, Fullerton, CA). Plasma immunoreactive insulin and glucagon concentrations were measured using double antibody radioimmunoassay kits (insulin; Diagnostic System Labs, Webster, TX) (glucagon; Linco, St. Charles, MO). Plasma lactate concentrations were measured by using the lactate dehydrogenase method (13). Plasma concentrations of fatty acids were determined using a microfluorimetric method (14).

Liquid chromatography tandem mass spectrometry. Plasma acetaminophen-glucuronide ^{13}C atom percent enrichment (APE) was determined by liquid chromatography tandem mass spectrometry after deproteinization with methanol (1:5), lyophilizing and reconstitution in methanol/H_2O (1:1). The analysis was performed with Perkin-Elmer Sciex API 3000 Electronspray Interface Tandem Mass Spectrometry (ESI/MS/MS) (Perkin-Elmer, Foster City, CA) interfaced with a Hewlett-Packard 1090 liquid chromatograph (Hewlett-Packard, Palo Alto, CA). Multiple reactions monitoring was adapted in which Tylenol glucuronide and its ^{13}C isotopic form were monitored through the transition from precursor to product ion (m/z 326 to m/z 113 and m/z 327 to m/z 114, respectively) in negative ion mode. Liquid chromatography conditions were 50:50 isocratic (A = 5% methanol/10 mmol/l ammonia acetate, B = 95% methanol/10 mmol/l ammonia acetate) with a flow rate of 200 μl/min. A sample with natural abundance ^{13}C was included with each batch. Peak areas were taken for M = 326/113 and (M + 1) = 327/114, and the ^{13}C APE in the sample was calculated as follows: $1/[1 + M + 1)/M_{sample} + (M = 1)/M_{natural}]$. The ^{13}C APE for the natural abundance samples was 5.8%.

Plasma fructose concentrations were measured after the addition of 0.1 ml of 0.55 mmol/l mannitol per 0.2 ml plasma as the internal standard and deproteinization with equal volume of 0.3 N barium hydroxide and 0.3 N zinc sulfate. After drying, the samples were derivatized with acetic anhydride and pyridine, and plasma fructose concentrations were measured using GC-MS with electron impact ionization. Ions with m/z 275 and 361 were monitored for fructose and mannitol, respectively, in the selective ion-monitoring mode.

Calculations and data analysis. The method for estimating rates of hepatic glycogen synthesis and simultaneous breakdown is based on measuring initial total glycogen concentration (^{13}C + ^{12}C) and subsequent changes in C1 glycogen peak integral (PI) (ΔPI) (7). Briefly, the rate of hepatic accumulation of glycogen was assessed from the increase in ^{13}C-glycogen concentration during the initial [1-^{13}C]glucose infusion (0–120 min). The increase in total liver glycogen concentration (ΔGly) for each 15-min interval is as follows:

[ΔPI · ^{13}C glycogen concentration · 100]/[(b · plasma glucose ^{13}C enrichment over baseline) + 1.1],

where b is the dilution factor representing the fraction of UDP-glucose formed by the direct pathway. The fraction of UDP-glucose formed from the direct pathway of glycogen synthesis is determined from the ^{13}C enrichment in C1 and C6 of plasma glucose and acetaminophen-glucuronide and calculated as follows:

b = (plasma glucuronide ^{13}C1 – ^{13}C6 enrichment)/(plasma glucose ^{13}C1 – ^{13}C6 enrichment).

Because the increase in the total liver glycogen concentration was linear from time 60–120 min during the infusion of [1-^{13}C]glucose, the hepatic accumulation of glycogen can be assessed from the slope of the line in a plot of the total hepatic glycogen concentration versus time. A minimal rate of simultaneous glycogen breakdown, i.e., only the fraction of glycogen that escapes the hexose-1-phosphate pool, was estimated by comparing the observed change in the ^{13}C1 glycogen concentration during the chase period (120–240 min) with the predicted change in ^{13}C1 glycogen, when assuming that constant glycogen synthesis is without breakdown. The observed change in the ^{13}C1 glycogen concentration is given as ΔPI · ^{13}C glycogen concentration, whereas the predicted change in ^{13}C1 glycogen during each 15-min time interval of the chase period is given as follows:

Δtime · rate of glycogen synthesis · [b · (^{13}C plasma glucose enrichment) + 1.1]/100.

The difference between the predicted and the observed change in ^{13}C1 glycogen represents an estimate for the amount of ^{13}C1 glycogen that is broken down during each 15-min time interval. The mean rate of ^{13}C1 glycogenolysis is obtained by the slope of the line from the plot of the difference between the predicted and observed changes in ^{13}C1 glycogen versus time. The rate of total glycogen breakdown ([^{13}C + ^{12}C]glycogen) is obtained by dividing this slope with the maximum plasma ^{13}C UDP-glucose enrichment [(b · maximum plasma ^{13}C glucose enrichment) + 1.1]. The relative percent hepatic glycogen turnover is then calculated as the ratio of the rate of glycogenolysis to the rate of hepatic glycogen synthesis × 100%; and the net rate of hepatic glycogen synthesis is calculated as the difference between the rates of hepatic glycogen accumulation and glycogen breakdown (7).

All data are presented as means ± SE. Linear regressions were calculated by the method of the least squares, and differences between the control and the fructose studies were assessed by paired t tests.

RESULTS

Plasma substrate and hormone concentrations. Plasma concentrations of glucose (control 6.0 ± 0.2 and fructose 5.8 ± 0.2 mmol/l) and insulin (control 48 ± 6 and fructose 42 ± 6 pmol/l) were similar between the experimental protocols in the overnight-fasted state. During the euglycemic-hyperinsulinemic clamp, plasma glucose and insulin concentrations were maintained at similar steady-state concentrations between the fructose and control studies (plasma glucose control 6.8 ± 0.1 and fructose 6.8 ± 0.1 mmol/l; plasma insulin control 354 ± 6 and fructose 312 ± 30 pmol/l; $P < 0.001$ vs. basal) (Fig. 1). There were no differences between plasma glucagon concentrations in the control and fructose studies at baseline (control 49 ± 5 and fructose 51 ± 5 ng/l) or during the clamp (control 33 ± 1 and fructose fast 35 ± 1 ng/l; P = 0.23). Fasting plasma concentrations of fatty acids (control 611 ± 35 and fructose 551 ± 17 μmol/l) were suppressed equally during the clamps studies (control 147 ± 8

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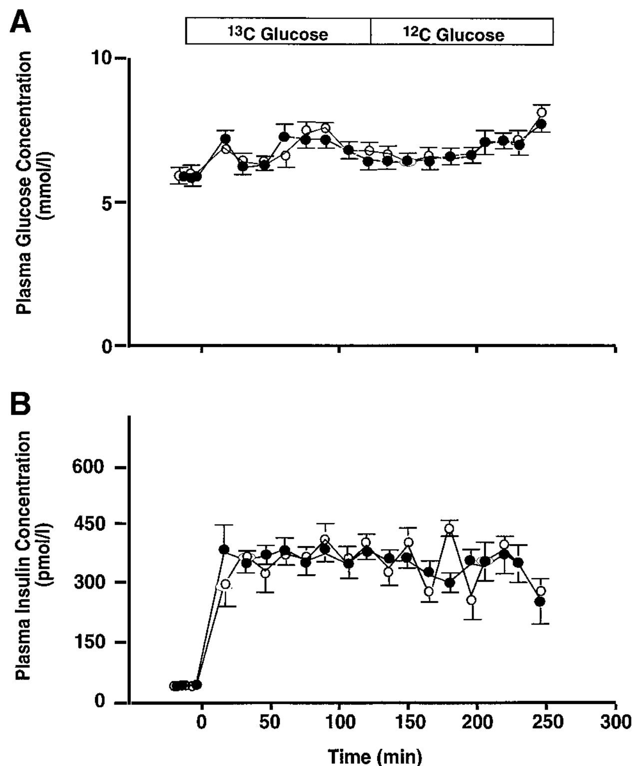

FIG. 1. *A*: Plasma concentrations of glucose (mmol/l). *B*: Plasma insulin concentrations (pmol/l) under euglycemic (5 mmol/l) hyperinsulinemic (400 pmol/l) conditions. ○, Control studies; ●, fructose studies.

and fructose 161 ± 6 μmol/l; $P = 0.14$). There were no differences in plasma lactate concentrations between the control (0.64 ± 0.06 mmol/l) and the fructose (0.50 ± 0.06 mmol/l) studies before the experiments, and plasma lactate increased similarly in both protocols during the clamp (control 1.06 ± 0.14 and fructose 1.10 ± 0.06 mmol/l; $P < 0.005$ vs. basal). Basal plasma fructose concentrations were 128 ± 5 μmol/l and increased during the fructose infusion by approximately a factor of 2 to 276 ± 9 μmol/l ($P < 0.0001$).

Glucose infusion rates. The mean glucose infusion rates from 60 to 240 min were similar in the two studies (control 45.5 ± 3.8 and fructose 45.7 ± 3.8 $\mu mol \cdot kg^{-1} \cdot min^{-1}$). The fructose infusion rate was 3.5 $\mu mol \cdot kg^{-1} \cdot min^{-1}$.

Pathways of hepatic glycogen synthesis. An estimate of the percent contribution of the direct pathway of hepatic glycogen synthesis was obtained by the ratio of plasma ^{13}C enrichments in glucose and acetaminophen-glucuronide. The percent contribution of the direct pathway to hepatic glycogen synthesis was similar in the control (63 ± 5%) and the fructose (57 ± 2%, $P = 0.10$) studies.

Basal hepatic glycogen concentration and estimated rates of glycogen synthase flux, glycogen phosphorylase flux, and net hepatic glycogen synthesis. Fasting liver glycogen concentrations were similar before each clamp study (control 259 ± 25 and fructose 255 ± 15 mmol/l). Total liver glycogen concentrations increased in a linear fashion 60–120 min during both study protocols (control $r = 0.99$ and fructose $r = 0.99$) (Fig. 2). Figure 3 depicts the mean increase in the integrated peak intensity from C1 liver glycogen in the ^{13}C NMR spectra in the control and fructose infusion studies, respectively. The peak intensity at the beginning of the chase period was set to one in all subjects. Note that the 120-min time point, when the [1-^{13}C]glucose infusion was replaced by the ^{12}C glucose chase, was set to time 0 in Fig. 3. It should also be noted that the variability indicated by the SE bars is primarily caused by differences between subjects, not intraindividual variations. The intraindividual variations caused by noise in the NMR measurements were <10% in the basal state and remained constant, i.e., approximately ±0.1 peak intensity unit, throughout each study. The C1 glycogen peak intensity reached a peak at the end of the [1-^{13}C]glucose infusion and declined in a similar fashion in the control and fructose studies (Fig. 3). Also in Fig. 3*A* and *B* are the predicted mean increases in C1 glycogen peak intensity calculated from the measured plasma glucose ^{13}C enrichment during the chase period as described in RESEARCH DESIGN AND METHODS. In both the control and the fructose studies, a measurable increase in C1 glycogen peak intensity is predicted in the absence of glycogen turnover. In the control studies, the flux through glycogen synthase was 0.31 ± 0.06 mmol/l per min and through glycogen phosphorylase was 0.17 ± 0.04 mmol/l per min (~55% of the glycogen synthase flux). Consequently, rates of net hepatic glycogen synthesis were relatively low (0.14 ± 0.05 mmol/l per min), which is consistent with our previous observations under the same conditions (7). During the fructose infusion, flux through glycogen synthase increased by a factor of 2.5 to 0.79 ± 0.16 mmol/l per min ($P = 0.018$ vs. control) (Fig. 4), whereas flux through glycogen phosphorylase was unchanged (0.24 ± 0.06 mmol/l per min; $P = 0.16$ vs. control) (Fig. 4). This stimulating effect of fructose on glycogen synthase flux resulted in a threefold increase in the net rate of hepatic glycogen synthesis (0.54 ± 0.12 mmol/l per min; $P = 0.008$ vs. control) (Fig. 4) and a tendency toward a decrease in the percent hepatic glycogen cycling as estimated by the ratio of glycogen synthase flux and glycogen phosphorylase flux (control 57 ± 14 and fructose 33 ± 6%, $P = 0.18$).

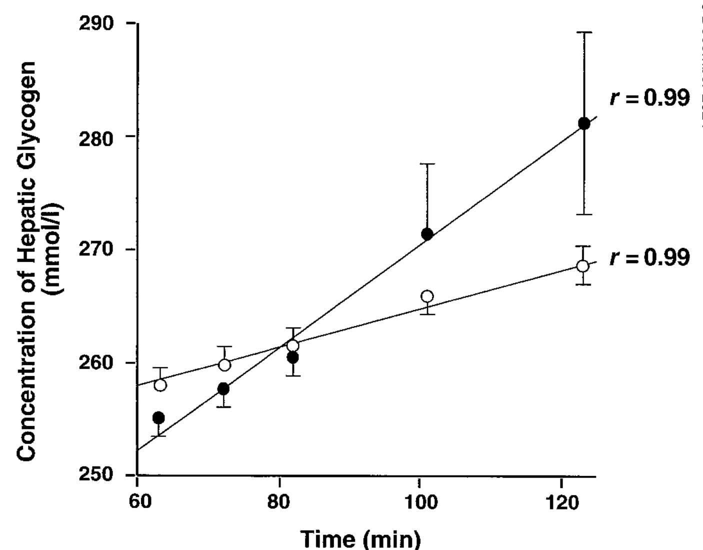

FIG. 2. Rates of net hepatic glycogen synthesis in humans under euglycemic (5 mmol/l) hyperinsulinemic (400 pmol/l) conditions. □, Control studies; ■, fructose studies.

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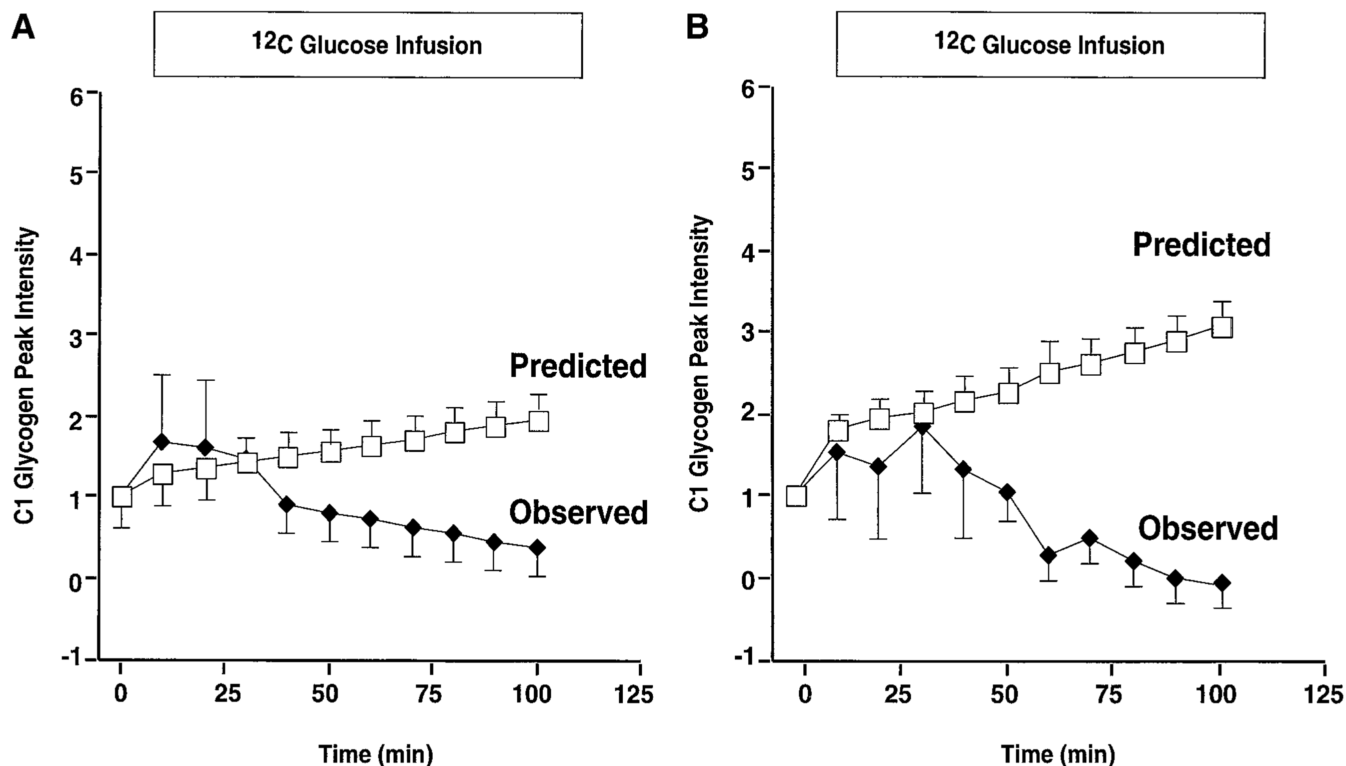

FIG. 3. Mean increment in observed carbon-1 (C1) glycogen peak area and predicted C1 glycogen peak area in the control studies (*A*) and the fructose infusion studies (*B*) under euglycemic (5 mmol/l) hyperinsulinemic (400 pmol/l) conditions.

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DISCUSSION

In the present study, we show that a relatively low-dose (~9 g) infusion of fructose during a euglycemic-hyperinsulinemic clamp, which raised plasma fructose concentrations from 130 to 280 μmol/l, resulted in a 2.5-fold stimulation of flux through glycogen synthase without affecting glycogen phosphorylase flux, thereby resulting in a threefold increase in rates of net hepatic glycogen synthesis when compared with the control studies, where no fructose was infused. These data are consistent with the findings of a study performed in dogs in which small amounts of fructose were infused into the portal circulation during a hyperglycemic-hyperinsulinemic clamp (5). The rate of hepatic glycogen synthesis was estimated from the glycogen concentration in the liver tissue obtained at the end of the study and the net incorporation of ^{3}H into liver glycogen. Using this approach, the rate of hepatic glycogen synthesis was found to be threefold higher when compared with historical controls. Because the rate of glycogen synthesis was calculated indirectly and these studies were performed with three ascending infusion rates of fructose, it is difficult to compare directly the rates of glycogen synthesis in these dog studies with our study (5).

There were no differences in rates of glucose infusion during the control and fructose studies, although rates of hepatic glucose uptake increased significantly in the fructose studies. The increase in hepatic glucose uptake during the fructose studies (from 0.31 mmol/l per min [~7.4 $\mu mol \cdot kg^{-1} \cdot min^{-1}$] to 0.79 mmol/l per min [~19 $\mu mol \cdot kg^{-1} \cdot min^{-1}$] would account for ~12 or ~2 $mg \cdot kg^{-1} \cdot min^{-1}$. The infusion rate of fructose was 3.5 ± 0.2 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, which could account for approximately one-third of the increase in hepatic glucose uptake. The remaining would be too small to detect in our glucose infusion rates.

Shiota et al. (5) estimated the amount of glycogen synthesized by the direct pathway by dividing the ^{3}H radioactivity in liver glycogen by the average ^{3}H specific activity in arterial plasma glucose during the hyperglycemic clamp and found it to be ~90% compared with 54–58% in historical control studies (15,16). In contrast, we found that the direct pathway accounted for ~60% of hepatic glycogen synthesis in the control study, which is similar to our previous results in humans (7,17), and that the contribution of the direct pathway of liver glycogen synthesis was unchanged by the infusion of fructose. It is unclear why these results differ, but this may be attributed to differences in the protocols, as our study was conducted under conditions of euglycemia (6.8 mmol/l), and the dog studies were performed under conditions of hyperglycemia (12.5 mmol/l) with a graded infusion of fructose. In addition, plasma insulin concentrations in our human studies were ~330 pmol/l, which was 1.7-fold higher than those in the dog studies (195 pmol/l), and we did not replace basal glucagon levels, as was done in the dog studies.

Interestingly, fructose caused a 2.5-fold increase in flux through glycogen synthase without any changes in the percent contribution of flux through the direct or indirect pathways of glycogen synthesis. These data suggest that fructose regulates flux into liver glycogen not only at glucokinase but also possibly at the level of glycogen synthase. In contrast to the marked stimulatory effects of fructose on glycogen synthase flux, we found that infusion of small amounts of fructose had no significant effects on glycogen phosphorylase activity. The regulation of phos-

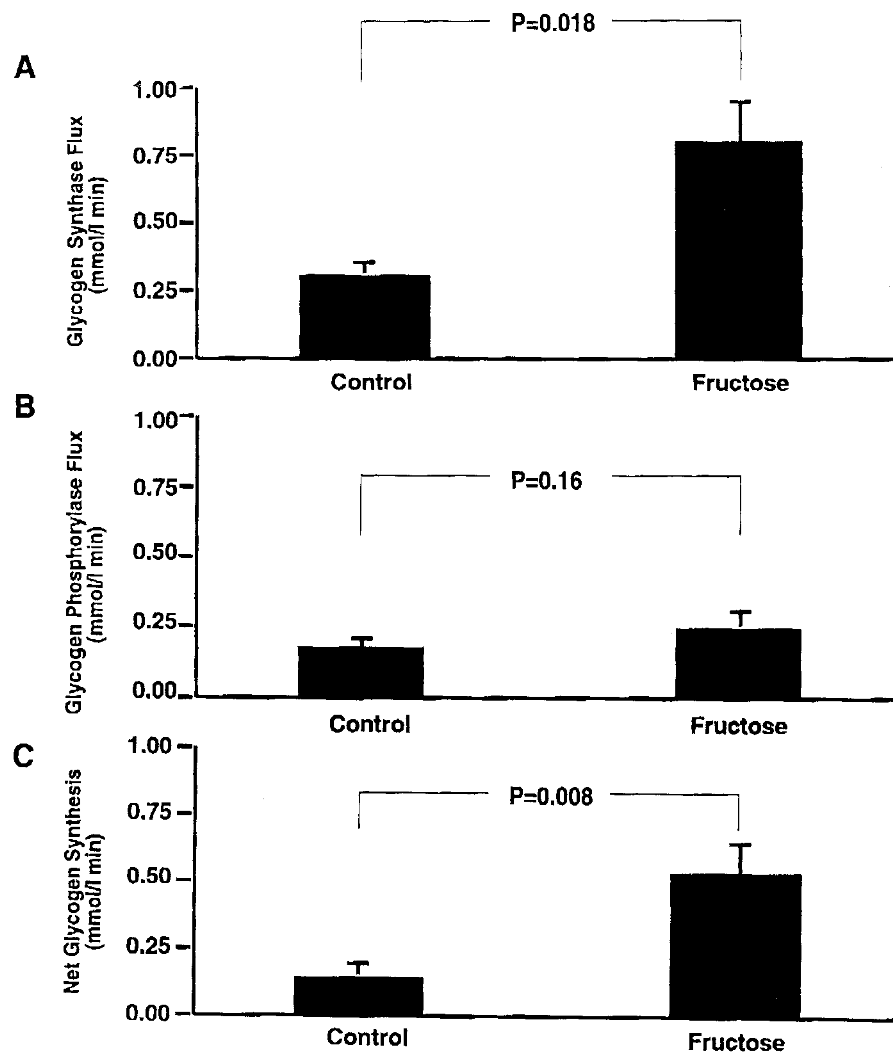

FIG. 4. Estimated rates of glycogen synthase flux (*A*), glycogen phosphorylase flux (*B*), and net hepatic glycogen synthesis (*C*) in control and fructose studies under euglycemic (5 mmol/l) hyperinsulinemic (400 pmol/l) conditions.

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phorylase activity by fructose is controversial. In liver extract studies, fructose-1-P has been found to inhibit phosphorylase phosphatase and to activate phosphorylase (18,19), whereas in the perfused liver, fructose has been shown to stimulate glycogen synthesis by inhibition of glycogen phosphorylase activity (20).

In summary, these are the first studies to demonstrate directly that small amounts of fructose can have a profound impact on stimulating net hepatic glycogen synthesis in humans. Furthermore, these studies reveal that the mechanism by which this occurs is through stimulation of glycogen synthase flux by 2.5-fold, with no significant effect to inhibit glycogen phosphorylase flux. Because net hepatic glycogen synthesis (21,22) has been shown to be diminished in patients with poorly controlled type 1 and type 2 diabetes, stimulation of net hepatic glycogen synthesis by this mechanism may be of potential therapeutic value.

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