Nutrient-Gene Interactions

An Acute Increase in Fructose Concentration Increases Hepatic Glucose-6-Phosphatase mRNA via Mechanisms That Are Independent of Glycogen Synthase Kinase-3 in Rats1,2

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ABSTRACT It appears that low amounts of fructose improve, whereas increased concentrations impair glucose tolerance and hepatic glucose metabolism. In this study, we compared directly the effects of low vs. high portal vein fructose concentrations on hepatic glucose metabolism in rats, using glucose-6-phosphatase gene expression as an endpoint. In the control group (C; n = 7), pancreatic clamps were performed using somatostatin and replacement of insulin such that basal glucose levels were maintained. In the experimental groups (n = 8/group), hyperglycemic, hyperinsulinemic pancreatic clamps were performed in which glucose (G) or glucose + fructose was infused into a jejunal vein. Fructose was infused to achieve either low (F1; <0.3 mmol/L) or high (F2; >1.0 mmol/L) portal vein concentrations. Total sugar load to the liver was equalized among the 3 experimental groups. Compared with C, liver glucose-6-phosphatase catalytic subunit mRNA was reduced by ~55% in G and F1, whereas it was increased ~180% in F2. F2 did not differentially affect glucose-6-phosphate translocase or phosphoenolpyruvate carboxykinase mRNA levels in liver, nor kidney glucose-6-phosphatase catalytic subunit mRNA. Livers from the F2 group were characterized by an accumulation of pentose phosphate intermediates and reduced phosphorylation of glycogen synthase kinase-3 (active form). However, in separate studies (n = 5/group), the infusion of a glycogen synthase kinase-3 inhibitor did not prevent the effects of F2 on glucose-6-phosphatase gene expression. We hypothesize that elevated fructose concentrations, similar to levels achieved after ingestion of sucrose- or fructose-enriched meals, initiate signals within the liver that elicit selective changes in hepatic gene expression. J. Nutr. 134: 545–551, 2004.

KEY WORDS: • gene expression • liver • simple sugars

There appears to be growing interest and concern over recent upward trends in sucrose and fructose consumption (1–7). The bulk of fructose metabolism occurs in the liver, and the presence of fructose stimulates glucose uptake by the liver (5). Thus, the liver may be particularly vulnerable to diets enriched in sucrose or fructose. We examined the effect of sucrose-enriched diets on hepatic glucose metabolism in rats to determine the potential of sucrose and its constituent monosaccharides, fructose and glucose, to produce hepatic impairments that characterize prediabetic states and type 2 diabetes. In rats, diets enriched in sucrose reduce the ability of insulin to suppress glucose production in vivo, reduce the ability of insulin to suppress gluconeogenesis in perfused liver, and increase the capacity for gluconeogenesis in vivo and in vitro (8–11). These diet-induced adaptations occur rapidly (1 wk) and independently of changes in body composition, and are accompanied by increased hepatic lipids (8).


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Low doses of fructose that achieve portal vein concentrations ≤ 0.3 mmol/L, stimulate glucose uptake by the liver (12), increase hepatic glycogen synthesis (13), and reduce the glycemic response to a glucose tolerance test (14). These beneficial effects of low quantities of fructose are in contrast to the impairments in hepatic glucose metabolism and insulin action that occur after exposure to diets enriched in sucrose or fructose (8,15) or when fructose was infused at relatively high rates (16), i.e., conditions that elicit concentrations of fructose that exceed 0.5 mmol/L. A direct comparison of the effects of low vs. high dose fructose infusion on hepatic glucose metabolism has not been performed in vivo. Thus, the first aim of the present study was to compare directly the effects of low vs. high portal vein fructose concentrations on hepatic glucose metabolism, using glucose-6-phosphatase gene expression as an endpoint.

Glucose-6-phosphatase is a multicomponent enzyme that is tightly associated with the endoplasmic reticular membrane and catalyzes the dephosphorylation of glucose-6-phosphate (G6P), the terminal step of glycogenolysis and gluconeogenesis.

4 Abbreviations used: C, control basal clamp group; F1, hyperglycemic, hyperinsulinemic clamp group with jejunal vein glucose infusion and low fructose infusion; F2, hyperglycemic, hyperinsulinemic clamp group with jejunal vein glucose infusion and high fructose infusion; G, hyperglycemic, hyperinsulinemic clamp group with jejunal vein glucose infusion; G6P, glucose-6-phosphate;
esis (17,18). G6P hydrolysis appears to involve a G6P translocase (G6PT) protein, which functions to transport G6P across the endoplasmic reticulum, and a catalytic subunit (G6Pase), located on the luminal side of the endoplasmic reticulum (17,18). In a previous study, we demonstrated that a sucrose-enriched diet (68% of energy from sucrose) increased the amount of G6Pase protein when provided to rats for only 1 wk (11). In addition, ingestion of a single, sucrose-enriched meal increased the expression of the gene encoding G6Pase above levels observed in food-deprived rats (19). This increase in G6Pase gene expression was accompanied by reduced phosphorylation of glycogen synthase kinase-3 (GSK3). GSK3 is a serine/threonine kinase originally identified by virtue of its ability to phosphorylate and inactivate glycogen synthase (20). The selective inhibition of GSK3 was shown to reduce the expression of both the G6Pase and phosphoenolpyruvate carboxykinase (PEPCK) genes in hepatoma cells (21). Thus, it was postulated that the postprandial environment after ingestion of a sucrose-enriched meal provoked the upregulation of G6Pase mRNA in part via cellular changes that reduce phosphorylation of, and therefore activate, GSK3. The second aim of the present study was to test this hypothesis using a variation of the pancreatic-glucose clamp technique and a commercially available inhibitor of GSK3.

MATERIALS AND METHODS

Animals. Male Wistar Crl:(WI)BR rats (Charles River) weighing ~180 g upon arrival were provided free access to a high-starch diet and water. The diet was formulated by Research Diets and contained the following: casein, 200 g/kg; taurine, 3 g/kg; cornstarch, 500 g/kg; maltodextrin 10, 150 g/kg; cellulose, 50 g/kg; corn oil, 50 g/kg; salt mix, 35 g/kg; vitamin mix, 10 mg/kg; and choline bitartrate, 2 mg/kg (19). Rats were housed individually in a temperature- and humidity-controlled environment with a 12-h light:dark cycle. Surgical procedures were performed after 1 wk of acclimatization. All procedures were reviewed and approved by the UCHSC institutional animal care committee.

Surgical procedures. Rats were deprived of food overnight and then were anesthetized using ketamine (50 mg/kg), xylazine (10 mg/kg), and acepromazine (5 mg/kg). Catheters were then implanted into the femoral vein to supply heparinized saline and were exteriorized on the dorsal pad. The abdomen was opened, and simultaneous arterial and portal vein blood samples were obtained (terminal samples), and portions of the liver and kidney were removed and processed for subsequent analyses. The gastrocnemius muscle was also removed and immediately frozen for subsequent analysis.

Experimental procedures. On the day of study, extensions were added to catheters of rats deprived of food for 6–8 h. Rats were allowed to rest for 30 min and then a baseline blood sample was taken. To investigate the effects of the portal vein fructose concentration, rats underwent 1 of 4 experimental protocols. In all of these protocols, somatostatin [1.2 μg/(kg · min)] was infused into the jugular vein to inhibit endogenous insulin secretion. In protocol 1 (C), a pancreatic clamp was performed in which insulin was infused into the jejunal vein catheter at rates (4.2 ± 0.3 pmol/(kg · min)) designed to maintain glucose levels at baseline values. This protocol was used to control for effects of time and somatostatin. In the remaining protocols (2–4), the insulin infusion rate was 16 pmol/(kg · min). In protocol 2 (G), a hyperglycemic, hyperinsulinemic clamp was performed in which glucose was infused at a constant rate into the jejunal vein [20 μmol/(kg · min)] and at a variable rate into the jugular vein to establish moderate hyperglycemia. This protocol was used to mimic the postprandial environment of hyperinsulinemia, hyperglycemia, and portal vein glucose levels in excess of arterial levels. In protocols 3 and 4, arterial glucose levels were equalized by the infusion of a fructose infusion into the jejunal vein that was designed to elevate portal vein fructose concentrations to <0.3 mmol/L (F1) or >1.0 mmol/L (F2). In F1 and F2, arterial glucose levels were adjusted in an attempt to equalize the total hepatic sugar load (glucose + fructose) with that of G. Thus, F1 and F2 were designed to examine the effects of low vs. high fructose concentrations on hepatic gene expression. All protocols were 2 h in duration.

To investigate the hypothesis that activation of glycogen synthase kinase mediated the effects of high portal vein fructose concentrations, studies identical to those described above were carried out (C, G, F1, F2; n = 5/group) in which an inhibitor of glycogen synthase kinase was infused into the jugular vein [50 μg/(kg · min)]. The infusion of the inhibitor was initiated 2 h before the start of the clamp. The inhibitor was a thiazolidinone analog that acts as a selective, non-ATP competitive inhibitor of GSK3β (4-benzyl-2-methyl-1,2,4-thiazolidinidine-3,5-dione; EMD Biosciences) (23). At the end of the 2 h experimental period, rats were anesthetized with methohexital (70 mg/kg), placed on a heating pad. The abdomen was opened, simultaneous arterial and portal vein blood samples were obtained (terminal samples), and portions of the liver and kidney were removed and processed for subsequent analyses. The gastrocnemius muscle was also removed and immediately frozen for subsequent analysis.

Processing and analysis of blood samples. All blood samples were immediately centrifuged at 800 × g for 15 min and the plasma used either for immediate analysis of glucose (Beckman Glucose Analyzer) or stored at −80°C for later analysis of fructose (Sigma Kit FA-20), insulin (Linco Research, RI313K) or glucagon (Linco, GL-32K). Insulin and glucagon concentrations were measured on the baseline and terminal samples (120-min time point before anesthesia and after anesthesia). Fructose, after removal of glucose using SigmaGel (SPSS) analysis software. The ratio between the target mRNA and 18S rRNA was calculated. Data are presented as the fold change in the target/18S ratio from the control group (protocol 1 or C). Each PCR reaction was performed in duplicate on two individual preparations of reverse-transcribed cDNA.

Tissue preparation, immunoprecipitation and Western blotting. Freshly removed liver and kidney were homogenized on ice in a buffer containing 20 mmol/L HEPES, pH 7.4, 1% Triton, 10% glycerol, 2 mmol/L EGTA, 1 mmol/L sodium vanadate, 2 mmol/L dithiothreitol, 1 mmol/L phenylmethylsulfonyl fluoride, 50 mmol/L β-glycerophosphate, 3 mmol/L benzamidine, 10 mmol/L leupeptin, 5 mmol/L pep-

G6Pase, glucose-6-phosphatase catalytic subunit; G6PT, glucose-6-phosphate translocase; GSK3, glycogen synthase kinase-3; IRS1, insulin receptor substrate-1; IRS2, insulin receptor substrate-2; PEPCK, phosphoenolpyruvate carboxykinase; p-GSK3, phospho-GSK3; PI3-kinase, phosphatidylinositol 3-kinase; PKB, protein kinase B; Akt; PKB, phospho-PKB; X5P, xylulose-5-phosphate.
Glucose, fructose and insulin concentrations that were achieved during the clamps are provided in Table 1. Arterial glucose concentrations were varied in G, F1, and F2 in an attempt to equalize the total sugar load (glucose + fructose) reaching the liver. Assuming a distribution of blood flow to the liver of 20% in the artery and 80% in the portal vein (28), the mean sugar load to the liver was 10.8 mmol/L in G, 10.9 mmol/L in F1, and 11.2 mmol/L in F2. The decrease in fructose concentration between arterial and portal vein samples (Table 1) illustrates the extent of hepatic extraction of this nutrient. The time-weighted, mean peripheral glucose infusion rate over the last 30 min of the clamp was 73 ± 7 μmol/(kg · min) in C, 121 ± 11 μmol/(kg · min) in G, 116 ± 15 μmol/(kg · min) in F1, and 110 ± 12 μmol/(kg · min) in F2. The CV in the glucose infusion rate over the final 30 min did not differ among the groups (pooled mean: 2.2 ± 0.1%).

**Liver intermediates.** Liver G6Pase was significantly greater in G (143 ± 11 nmol/g), F1 (158 ± 15 nmol/g), and F2 (163 ± 14 nmol/g) compared with C (59 ± 4 nmol/g). Accumulation of pentose phosphate intermediates, such as X5P, occurs when the rate of carbohydrate utilization by the liver exceeds the rates of glycogen synthesis and glycolysis (26). Thus, in the present study, we used X5P as a marker for the extent of hepatic sugar removal by the liver. X5P was 2.2 ± 0.3 mmol/L in G, 10.2 ± 1.2 mmol/L in F1, 15.3 ± 1.8 mmol/L in F1, and 49.1 ± 4.2 mmol/L in F2.

**RNA analysis by RT-PCR.** Compared with C, liver G6Pase mRNA was ~55% lower in G and F1 (P < 0.05), whereas it was 1.8-fold higher (P < 0.05) in F2 (Fig. 1). Consequently, G6Pase mRNA in F2 was ~3-fold higher compared with G and F1 (Fig. 1). In contrast, liver G6PT mRNA did not differ among any of the groups, and both liver PEPCK mRNA and kidney G6Pase mRNA were reduced by ~50% in G, F1, and F2 compared with C (data not shown).

**Signal proteins.** As in our previous report (19), we measured several proximal insulin signaling steps to determine whether the upregulation of liver G6Pase mRNA in response to F2 involved impairments in insulin signaling. Several previous studies documented the dominant, suppressive role of insulin in the regulation of liver G6Pase gene expression (29,30). Tyrosine phosphorylation of IRS-1, IRS-1 association with PI3-kinase, and phosphorylation of PKB did not differ among G, F1, and F2 (Fig. 2). Similar results were observed for IRS2 phosphorylation and IRS2 association with PI3-kinase (data not shown). However, serine phosphorylation of GSK3 was significantly lower in F2 compared with G and F1 (Fig. 2).

### TABLE 1

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<thead>
<tr>
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<th>C</th>
<th>G</th>
<th>F1</th>
<th>F2</th>
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<tr>
<td>Arterial glucose, mmol/L</td>
<td>7.3 ± 0.5a</td>
<td>9.5 ± 0.3b</td>
<td>9.4 ± 0.5b</td>
<td>9.1 ± 0.3b</td>
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<td>Arterial fructose, mmol/L</td>
<td>ND</td>
<td>ND</td>
<td>0.13 ± 0.02a</td>
<td>0.31 ± 0.03b</td>
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<td>Portal vein glucose, mmol/L</td>
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<td>11.1 ± 0.6b</td>
<td>10.9 ± 0.5b</td>
<td>10.4 ± 0.6b</td>
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<td>Portal vein fructose, mmol/L</td>
<td>ND</td>
<td>ND</td>
<td>0.3 ± 0.1a</td>
<td>1.2 ± 0.2b</td>
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<td>Arterial insulin, pmol/L</td>
<td>155 ± 14a</td>
<td>732 ± 61b</td>
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<td>Portal vein insulin, pmol/L</td>
<td>440 ± 35a</td>
<td>2020 ± 156b</td>
<td>1904 ± 164b</td>
<td>2110 ± 182b</td>
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1. Values are means ± SEM, n = 7–9. Means in a row without a common letter differ, P < 0.05. ND, not detected.
2. Arterial glucose and fructose concentrations represent the mean of 3 values taken during the final 30 min of the clamp.
3. Arterial insulin concentrations are based on a single sample taken at 120 min.
4. Portal vein concentrations are based on a single sample taken from anesthetized rats at the termination of the clamp.
**DISCUSSION**

Insulin suppresses G6Pase mRNA, and this effect appears to be dominant over the stimulatory effects of glucose, cAMP, and dexamethasone on G6Pase mRNA expression (29,30). The dominant, suppressive effects of insulin on G6Pase mRNA predict that the postprandial environment would lower the levels of G6Pase mRNA relative to those in food-deprived rats. However, in a previous study, the ingestion of a single, sucrose-enriched meal increased G6Pase mRNA above levels observed in food-deprived rats (19). In the present study, we sought to examine the regulation of G6Pase gene expression by the constituent monosaccharides of sucrose, glucose and fructose, under conditions in which insulin levels were strictly controlled. In addition, we sought to determine whether glycogen synthase kinase-3 mediated sucrose and fructose induction of G6Pase mRNA. The data demonstrate that when the portal vein fructose concentration was selectively elevated to levels achieved after ingestion of sucrose- or fructose-enriched meals, hepatic G6Pase gene expression was increased above levels observed in control rats. Further, the presence of an inhibitor of glycogen synthase kinase-3 did not prevent fructose-mediated induction of G6Pase mRNA.

Fructose stimulates glucose phosphorylation, uptake, and glycogen synthesis in the liver (12,13,33). This stimulatory effect occurs at fructose concentrations ≤ 0.2 mmol/L; thus low, “catalytic” concentrations of fructose can improve glucose tolerance (14). Sucrose- or fructose-enriched diets lead to fatty liver, hepatic insulin resistance, and upregulation of gluconeogenic genes.

**GSK3 inhibitor study.** Serine phosphorylation of GSK3 renders it inactive, and recent work demonstrated that selective inhibition of GSK3 activity reduced the expression of G6Pase mRNA (21). To examine the role of GSK3 in fructose-mediated induction of G6Pase gene expression, we performed a second series of clamps, on separate rats, in which an inhibitor of GSK3 was infused.

Energy intake before (pooled mean: 441 ± 29 kJ/d) and following surgery (pooled mean: 391 ± 29 kJ/d) did not differ among the groups (n = 5/group). Body weight on the day of study also did not differ among groups (pooled mean: 268 ± 18 g). Before the initiation of clamps, arterial glucose (7.3 ± 0.6 mmol/L in C, 7.1 ± 0.5 mmol/L in G, 7.5 ± 0.6 mmol/L in F1, and 7.6 ± 0.4 mmol/L in F2) and insulin (157 ± 17 pmol/L in C, 144 ± 16 pmol/L in G, 162 ± 14 pmol/L in F1, and 149 ± 17 pmol/L in F2) did not differ among groups. Glucagon concentrations also did not differ among groups, and fructose was undetectable in all groups (data not shown).

Glucose, fructose and insulin concentrations that were achieved during these clamps are provided in Table 2. X5P was 1.8 ± 0.4 nmol/g in C, 8.1 ± 0.7 nmol/g in G, 10.5 ± 1.1 nmol/g in F1, and 41.7 ± 3.7 nmol/g in F2. The efficacy of the inhibitor was assessed using glycogen synthase activity in liver and muscle because suppression of GSK3 activity is required for activation of this enzyme (20,31,32). Liver and gastrocnemius muscle glycogen synthase activity was significantly increased by the infusion of the inhibitor (Fig. 3). However, the presence of the inhibitor did not prevent the increase in G6Pase mRNA in response to F2 (Fig. 4). Similar to clamps performed in the absence of the inhibitor, liver G6PT mRNA did not differ among any of the groups, and both liver PEPCK mRNA and kidney G6Pase mRNA were reduced by ~60 and ~40%, respectively, in G, F1, and F2 compared with C (data not shown). In addition, IRS1-pY, IRS1-p85, and phospho-PKB (p-PKB) in the liver did not differ in the presence vs. absence of the inhibitor (data not shown).
genesis in rats (8,15), and acute, high dose fructose infusions impair insulin action in humans (16). It has been hypothesized that the high capacity for fructose uptake by the liver accounts for alterations in hepatic fuel stores and metabolism that result from high fructose concentrations (5,11,16,19). This hypothesis has not been tested directly. The present study compared the effects of low vs. high portal vein fructose concentrations on hepatic glucose metabolism using a modification of the glucose clamp technique and G6Pase gene expression as an endpoint. The selective elevation of portal vein fructose concentrations to values approaching those observed after ingestion of sucrose- or fructose-enriched meals, increased liver G6Pase gene expression. This fructose concentration, however, did not differentially affect kidney G6Pase nor liver G6PT and PEPCK gene expression. Finally, the presence of elevated portal vein glucose alone or in combination with low levels of fructose resulted in the suppression of G6Pase mRNA. These data support the notion that fructose concentrations that mimic those observed after ingestion of sucrose- or fructose-enriched meals, not only selectively influence the metabolism but also differentially affect the expression of key enzymes involved in glucose metabolism.

### TABLE 2

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<th>C</th>
<th>G</th>
<th>F1</th>
<th>F2</th>
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<tbody>
<tr>
<td>Arterial glucose, mmol/L</td>
<td>7.1 ± 0.6$^a$</td>
<td>9.8 ± 0.5$^b$</td>
<td>9.4 ± 0.4$^b$</td>
<td>9.0 ± 0.5$^b$</td>
</tr>
<tr>
<td>Arterial fructose, mmol/L</td>
<td>ND</td>
<td>ND</td>
<td>0.10 ± 0.02$^a$</td>
<td>0.39 ± 0.06$^b$</td>
</tr>
<tr>
<td>Portal vein glucose, mmol/L</td>
<td>7.0 ± 0.5$^a$</td>
<td>11.6 ± 0.5$^b$</td>
<td>11.1 ± 0.6$^b$</td>
<td>10.5 ± 0.7$^b$</td>
</tr>
<tr>
<td>Arterial insulin, pmol/L</td>
<td>167 ± 19$^a$</td>
<td>701 ± 68$^b$</td>
<td>711 ± 68$^b$</td>
<td>731 ± 53$^b$</td>
</tr>
<tr>
<td>Portal vein insulin, pmol/L</td>
<td>401 ± 39$^a$</td>
<td>1911 ± 183$^b$</td>
<td>1998 ± 185$^b$</td>
<td>1865 ± 161$^b$</td>
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1 Values are means ± SEM, n = 5. Means in a row without a common letter differ, P < 0.05. ND, not detected.
2 Arterial glucose and fructose concentrations represent the mean of 3 values taken during the final 30 min of the clamp.
3 Arterial insulin concentrations are based on a single sample taken at 120 min.
4 Portal vein insulin concentrations are based on a single sample taken from anesthetized rats at the termination of the clamp.

### FIGURE 3

Liver (upper panel) and muscle (lower panel) glycogen synthase activity levels in rats after a euglycemic, normoinsulinemic pancreatic clamp (C) or after hyperglycemic, hyperinsulinemic pancreatic clamps in which glucose (G) or glucose + fructose (F) was infused into a jejunal vein. F1 and F2 groups are distinguished by the rate of fructose infusion which was designed to increase portal vein fructose levels to either <0.3 mmol/L or >1.0 mmol/L, respectively. A glycogen synthase kinase-3 inhibitor was infused in the plus inhibitor group as described in the Materials and Methods. Values are means ± SEM, n = 5–8. Bars without a common letter differ, P < 0.05.

### FIGURE 4

G6Pase mRNA levels in rats after a euglycemic, normoinsulinemic pancreatic clamp in the presence of a glycogen synthase kinase-3 inhibitor (C) or after hyperglycemic, hyperinsulinemic pancreatic clamps in which glucose (G) or glucose + fructose (F) was infused into a jejunal vein in the presence of a glycogen synthase kinase-3 inhibitor. F1 and F2 groups are distinguished by the rate of fructose infusion, which was designed to increase portal vein fructose levels to either <0.3 mmol/L or >1.0 mmol/L, respectively. Data are the ratios of G6Pase to 18S. The top portion of the figure is a representative gel. Values are means ± SEM, n = 5, with C set to 1. Bars without a common letter differ, P < 0.05.
liver but also specific genes involved in hepatic glucose release. The reason(s) for the apparent sensitivity of the catalytic subunit of G6Pase to fructose regulation are presently under investigation.

In a previous study, a single, sucrose-enriched meal did not impair proximal insulin signaling steps in the liver but did reduce phosphorylation of GSK3 (19). In the present study, when the portal vein fructose concentration was increased to ~1 mmol/L, the proximal insulin signaling steps were also not impaired but phosphorylation of GSK3 was reduced. Phosphorylation of GSK3 leads to a reduction of activity. The selective inhibition of GSK3 activity reduced the expression of G6Pase gene expression in hepatoma cells (21). Thus, we reasoned that GSK3 may mediate, fructose-induced upregulation of G6Pase. However, the use of an inhibitor of GSK3 did not prevent fructose induction of G6Pase mRNA, nor did it alter the response of G6PT or PEPCk mRNA in any of the groups. Thus, fructose induction of G6Pase gene expression appears to occur independently of GSK3. The mechanisms responsible for the selective reduction of GSK3 phosphorylation observed in F2 and the downstream consequences of this are currently under study.

The inhibitor we employed is reported to be a highly selective, non-ATP competitive inhibitor of GSK3β (23). In the present study, we did not evaluate GSK3 activity directly, but instead used glycogen synthase activity to monitor the effectiveness of the inhibitor (31,32). Infusion of the inhibitor increased liver and muscle glycogen synthase activity similarly in all groups. If F2 were characterized by higher GSK3 activity, one might expect a greater increase of glycogen synthase activity in this group when the inhibitor was present. This was not the case, we cannot rule out the possibility that GSK3 remained higher in F2 compared with the other groups even in the presence of the inhibitor. Infusion of the inhibitor under conditions of euglycemia and normoinsulinemia (C) did not affect G6Pase gene expression. This is in contrast to the effects of selective inhibition of GSK3 in hepatoma cells, in which reduced G6Pase gene expression was observed (21). Thus, it appears that GSK3 activity in the liver in vivo may not contribute substantially to the regulation of G6Pase gene expression.

Xylulose-5-phosphate (X5P) was measured in the present study as a marker of carbohydrate utilization by the liver (19,34). The greater accumulation of X5P in F2 compared with the other groups is consistent with the high rate of fructose-1-phosphate formation that accompanies elevated fructose concentrations and the diversion of excess into the pentose phosphate pathway. X5P is an important signaling molecule, involved in the activation of protein phosphatase 2A and formation of fructose 2,6-bisphosphate (34). In addition, it was shown recently that X5P mediates glucose-induced activation of L-type pyruvate kinase and lipogenic gene expression (34). Xylitol infusion in vivo in the presence of basal insulin levels (35) or exposure of primary hepatocytes to xylitol in the absence of insulin (36) increased G6Pase gene expression. Whether X5P accumulation in response to F2 is a component of the selective changes in either G6Pase gene expression or the level of GSK3 phosphorylation is presently under investigation.

In conclusion, the present study demonstrates that the selective elevation of fructose concentrations to levels achieved after ingestion of a sucrose- or fructose-enriched meal, increases G6Pase gene expression above that observed under food-deprived conditions. Although the increased delivery of fructose to the liver also resulted in reduced phosphorylation of GSK3, this protein does not appear to be involved in fructose-mediated regulation of G6Pase mRNA.

LITERATURE CITED