Glucose-6-Phosphatase Activity Is Not Suppressed but the mRNA Level Is Increased by a Sucrose-Enriched Meal in Rats\textsuperscript{1,2}

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ABSTRACT  The expression of glucose-6-phosphatase (G6Pase) mRNA is repressed by insulin and stimulated by cAMP and dexamethasone, with the insulin effect dominant. Both lipids and glucose increase the expression of G6Pase mRNA under conditions in which insulin is either absent or at basal levels. The aim of the present study was to investigate dietary nutrient regulation of G6Pase mRNA and protein under postprandial conditions. Male rats (n = 6–8/group) were deprived of food for 48 h and then either remained food deprived (FD) or were refed diets containing 68% corn starch and 12% corn oil (ST; % energy), 68% sucrose and 12% corn oil (SU) or 35% corn starch and 45% corn oil (HF) for 3 h. Rats were anesthetized, blood was drawn from the portal vein, and the liver was removed and immediately processed for subsequent analyses. Energy intake over the 3-h refeeding period did not differ among groups (208 ± 25 kJ). Portal vein glucose and insulin were 5.0 ± 0.2 mmol/L and 90 ± 18 pmol/L, respectively, in FD rats and were not significantly different among the refed groups (14.5 ± 1.2 mmol/L and 1302 ± 154 pmol/L, respectively). Compared with the FD rats, G6Pase mRNA was ~50% lower in ST and HF groups, whereas it was ~1.6 fold higher in SU-refed rats (P < 0.05). G6Pase activity in whole liver homogenates was lower in ST and HF rats than in FD and SU rats. Insulin receptor substrate (IRS) phosphorylation, IRS-association with phosphatidylinositol 3 (PI3)-kinase and activation of protein kinase B (PKB) were not significantly different among the refed groups. However, glycogen synthase kinase-3\alpha phosphorylation was lower and cAMP response element binding protein (CREB) phosphorylation was higher in SU rats than in ST and HF refed groups. Thus, the postprandial environment after ingestion of sucrose appears to overcome the dominant effects of insulin on G6Pase mRNA, perhaps via cellular changes that reduce phosphorylation of, and therefore activate, glycogen synthase kinase-3\alpha.  J. Nutr. 133: 32–37, 2003.

KEY WORDS: • liver • glucose uptake • fructose • rats

The liver is an important site of postprandial glucose disposal, accounting for the removal of up to 30% of an oral glucose load (1–3). The liver is also critically involved in dietary lipid and amino acid uptakes (4), and the presence of either or both of these nutrients can reduce hepatic glucose uptake (4,5). The composition of ingested carbohydrate also influences hepatic glucose metabolism. For example, the presence of fructose increases the contribution of the liver to carbohydrate/sugar removal, due to the remarkably high hepatic extraction of fructose and the ability of fructose-1-phosphate to stimulate glucose phosphorylation/uptake (6–8). The liver therefore functions as a dietary energy buffer with the extent of nutrient uptake determined in part by the composition of nutrients delivered to this organ. Alterations in the composition of dietary macronutrients profoundly and rapidly changes hepatic glucose metabolism in rats (9–15) and humans (16,17). For example, the provision of diets enriched in corn oil or sucrose, which is hydrolyzed to fructose and glucose during absorption, to rats for 1 wk increased gluconeogenesis (9–12) and reduced the ability of insulin to suppress hepatic glucose production (13–15). Recent studies in humans also suggest that diets enriched in fat (41 and 83% of energy with a 2:2:1 ratio of saturated to monounsaturated to polyunsaturated lipids) or protein [1.25–2.41 g/(kg · d)] can reduce insulin’s suppression of glucose production and/or increase gluconeogenesis (16,17). Importantly, these adaptations in hepatic glucose metabolism occur before and independently of changes in body composition (i.e., adiposity) and the circulating metabolite and hormonal milieu (9,14,18). Presently, very little is known about the intrahepatic sites that respond to changes in the composition of ingested dietary nutrients.

Liver glucose-6-phosphatase (G6Pase)\textsuperscript{4} catalyzes the hydro-

\textsuperscript{1} A preliminary report of the data was given at the Annual Meeting of the American Diabetes Association, June 2002, San Francisco, CA [Pagliassotti, M. J., Wei, Y. & Bizeau, M. E. (2002) Dietary sucrose overcomes the dominant effect of insulin on glucose-6-phosphatase (G6Pase) in vivo. Diabetes 51(suppl. 2): A348 (abs.)]|348 (abs.)

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\textsuperscript{4} Abbreviations used: anti-PY, anti-phosphotyrosine; CREB, cAMP response element binding protein; FD, food deprived; GBP, glucose-6-phosphate; G6Pase, glucose-6-phosphatase; G6PT, glucose-6-phosphate translocase; GSK-3, glycose
ysis of glucose-6-phosphate (G6P) generated from glycolysis and gluconeogenesis (19–21). G6P hydrolysis appears to involve a G6P translocase (G6PT) protein, which functions to transport G6P across the endoplasmic reticulum, and a catalytic subunit, located on the luminal side of the endoplasmic reticulum (19–21). Studies in FAO hepatoma cells (22), primary hepatocytes (22–24) and conscious rats (23,26) have demonstrated that hyperglycemia can stimulate G6Pase catalytic subunit gene expression in the absence of insulin (in vitro) or in the presence of basal insulin levels (in vivo). Elevations in other nutrients, such as lipids, fructose, xylitol or glycerol, also increase hepatic G6Pase catalytic subunit gene expression (23,24,27). The extent to which nutrients affect the expression of catalytic subunit and/or G6PT mRNA when ingested, and thus in the presence of hyperinsulinemia, has not been determined. This is an important consideration because increased nutrient concentrations typically occur in the context of hyperinsulinemia, and insulin repression of catalytic subunit gene expression dominates over the stimulatory effects of glucocorticoids and glucose (28,29).

The aims of the present study were to determine the effects of diets enriched in corn oil or sucrose on the expression of the G6Pase catalytic subunit and G6PT mRNA in vivo, to ascertain whether acute intake of these diets influences the activity of G6Pase, and to identify putative mediators of dietary nutrient-induced changes in G6Pase mRNA and activity.

MATERIALS AND METHODS

Animals. Male Wistar rats (Charles River, Wilmington, MA) weighing ~180 g upon arrival were provided free access to a high starch diet and water. Rats were housed individually in a temperature- and humidity-controlled environment with a 12-h light:dark cycle. Experiments were performed after 1 wk of acclimatization. All procedures were reviewed and approved by the UCHSC institutional animal care committee.

Experimental procedures. Rats were deprived of food for 48 h and then either remained food deprived (FD, n = 6) or were provided free access to one of the following diets for 3 h: high starch diet (ST; 68% of energy from cornstarch, 20% from protein, 12% from corn oil, Table 1); high sucrose diet (SU; 7; Table 1); or high fat (HF; 8; Table 1); or high starch (ST; 68% of energy from cornstarch, 20% from protein, 12% from corn oil, Table 1); or high sucrose diet; HF, high fat diet.

1 Maltodextrin 10° is enzyme-converted cornstarch with a dextrose equivalence of 10%.
2 Salt and vitamin mixes prepared according to guidelines from the American Institute of Nutrition (30).

Hexamers (Life Technologies, Carlsbad, CA). Transcribed cDNA was subjected to duplex polymerase chain reaction (PCR) amplification using primers specific for the following genes: G6Pase catalytic subunit, 5′-AAA GAG AGT GTC GGC ATC AAT C-3′ (forward) and 5′-AAG AGG CTG CAA GAG AGT GTA G-3′ (reverse); G6PT, 5′-TGA CCA GTG ACT CCT CCA AG-3′ (forward) and 5′-ATC TTG GCG ATA TTT CG-3′ (reverse); and phosphoenolpyruvate carboxykinase (PEPCK), 5′-GCT GGC AGC ATG GGO TGT TTG TAG AG-3′ (forward) and 5′-CGA TGC GCA ACA ACA ACC CTCA ACA G-3′ (reverse) and a competitor/primer mix specific for 18S ribosomal RNA (Integrated DNA Technology, Coralville, IA for G6Pase; Ambion, Austin, TX for other primers). All PCR reactions were also run with DNAse-treated RNA that had not been subjected to RT. These samples did not yield any products. The PCR products were separated on 2% gels and visualized with ethidium bromide staining. A digital image of the gel was obtained with an Alphaimager2000 (Alpha Innotech, San Leandro, CA) and band intensities quantified using SigmaGel (SPSS, Chicago, IL) analysis software. The ratio between the target mRNA and 18S ribosomal RNA was calculated. Data are presented as the fold change in the target/18S ratio from the FD controls. Each PCR reaction was performed in duplicate on two individual preparations of reverse-transcribed cDNA.

Tissue preparation, immunoprecipitation, and Western blotting. Freshly removed liver (~0.5 g) was homogenized on ice in a buffer containing 20 mmol/L Hepes, pH 7.4, 1% Triton, 10% glycerol, 2 mmol/L EDTA, 1 mmol/L sodium vanadate, 2 mmol/L dithiothreitol, 1 mmol/L phenylmethylsulfonylfluoride, 50 mmol/L β-glycerophosphate, 3 mmol/L benzamidine, 10 μmol/L leupeptin, 5 μmol/L pepstatin and 10 mg/ml aprotinin. Samples were rotated for 30 min at 4°C and centrifuged at 20,000 x g for 1 h. Total protein concentration was determined by the Bradford method (31).

For immunoprecipitations, equivalent amounts of protein were incubated with antibodies against insulin receptor substrate-1 (IRS-1; Upstate Biotechnology, Lake Placid, NY) or IRS-2 (Upstate Biotechnology) followed by incubation with Protein G-agarose (Upstate Biotechnology). Immunoprecipitated proteins were resolved by SDS-PAGE, electrotransferred to Hybrid-P membranes (Amersham Pharmacia Biotech, Piscataway, NJ) and membranes incubated with antibodies against phosphotyrosine (anti-pY; BD Transduction Laboratories, Lexington, KY) or the p85α subunit of phosphatidylinositol 3-kinase (PI3-kinase; Upstate Biotechnology). Detection was performed using enhanced chemiluminescence reagents (Amer sham Biosciences, Piscataway, NJ) and band intensity was analyzed by optical density.

In some cases, protein amounts were determined without immu-

TABLE 1

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1 Diets were formulated by Research Diets (New Brunswick, NJ); ST, starch diet; SU, high sucrose diet; HF, high fat diet.

2 Maltodextrin 10° is enzyme-converted cornstarch with a dextrose equivalence of 10%.

3 Salt and vitamin mixes prepared according to guidelines from the American Institute of Nutrition (30).
Liver (-1 g) was removed and immediately homogenized on ice in 18 mL of 10 mmol/L Heps, 0.25 mol/L sucrose, pH 7.3. Microsomes were extracted by the procedures of Daniele et al. (32). Total protein was determined by the Bradford method (31); microsomal fractions were subjected to SDS-PAGE as described above and membranes incubated with antibodies against the p36 catalytic subunit of G6Pase (gift from Dr. Gilles Mithieux, Lyon Cedex, France) (32) or the p46 G6PT (gift from Dr. Gerald van de Werve, Montreal, Canada) (33). Membranes were analyzed as described above.

**G6Pase activity.** G6Pase activity was assessed on portions of intact microsomes as previously described (34,35). G6Pase activity in freshly prepared whole liver homogenates was determined immediately after preparation of the initial Heps/sucrose homogenate described above (34,35). Latency of mannose-6-phosphate was assessed on intact microsomes and whole liver homogenates as described previously (36,37).

**Analysis of liver intermediates.** A portion of freeze-clamped liver was used to determine the concentration of G6P (38), xylulose-5-phosphate (X5P) (39) and inorganic phosphate (40).

**Data analysis and statistics.** Latency of mannose-6-phosphate was calculated as: 100 - [100 × (mannose-6-phosphate activity in intact microsomes or liver homogenates)/(mannose-6-phosphate activity in microsomes or homogenates treated with 0.5% cholate)] (36,37). Data were analyzed by ANOVA using Bonferroni’s multiple comparison test to determine means that differed and by the non-parametric analysis of Kruskal-Wallis. The two analyses gave identical interpretations. Significance was set at P < 0.05. All data are reported as means ± SEM.

**RESULTS**

**Food intake, plasma glucose, fructose and insulin.** Energy intake over the 3-h feeding period did not differ among groups: 223.8 ± 35.1 kJ in ST, 198.3 ± 19.2 kJ in SU and 209.2 ± 23.4 kJ in HF. Portal vein plasma glucose and insulin concentrations were greater than in the FD rats but did not differ among the refed groups. Glucose concentration was 5.0 ± 0.2 mmol/L in FD, 15.1 ± 1.6 mmol/L in ST, 14.4 ± 0.8 mmol/L in SU and 14.1 ± 1.2 mmol/L in HF. Insulin concentration was 90 ± 18 pmol/L in FD, 1350 ± 170 pmol/L in ST, 1256 ± 120 pmol/L in SU and 1294 ± 168 pmol/L in HF. Portal vein fructose concentrations were detectable only in the group refeed the SU diet (1.4 ± 0.2 mmol/L).

**Liver intermediates.** Liver phosphate was significantly lower and X5P (a pentose phosphate pathway intermediate) was lower in rats refeed the SU diet compared with those refeed either the ST or HF diets (Table 2). Liver glucose-6-phosphate concentrations did not differ among the refed groups (Table 2).

**RNA analysis by RT-PCR.** Compared with food-deprived rats (FD), liver G6Pase catalytic subunit mRNA was 50% lower (P < 0.05) in rats refeed ST or HF, whereas it was ~1.6-fold higher (P < 0.05) in those refeed SU (Fig. 1 upper panel). Consequently, catalytic subunit mRNA in SU-fed rats was ~4-fold higher than in those fed ST and HF. Refeeding did not affect G6PT mRNA (Fig. 1 middle panel). Compared with FD-fed rats, PEPCK mRNA levels were ~50% lower in rats refeed ST (P = 0.02) and ~25% lower in those refeed SU (P = 0.24) or HF (P = 0.37) (Fig. 1 lower panel).

**Liver glucose-6-phosphatase (G6Pase) activity and Western blot analysis.** Microsomal G6Pase activity did not differ among FD rats [217 ± 18 nmol/(mg protein · min)], and those refed ST [208 ± 15 nmol/(mg protein · min)], SU [224 ± 16 nmol/(mg protein · min)] or HF [209 ± 13 nmol/(mg protein · min)]. There were also no differences in the amount of either the p36 catalytic subunit or the p46 G6PT microsomal proteins among groups (data not shown). In contrast, G6Pase activity in whole liver homogenates was 50% lower (P < 0.05) in rats refeed either ST or HF compared with the FD and SU groups (Fig. 2). The percentage latency of microsomes was 90% in all preparations and that in whole liver homogenates was 70 ± 4% with no differences among groups.

**Insulin signaling.** We measured several proximal insulin signaling steps to determine whether alterations in the regulation of G6Pase mRNA and activity after SU refeeding involved impairments in insulin action. However, IRS-1 tyrosine phosphorylation, IRS-1 association with the p85 subunit of phosphatidylinositol 3-kinase (PI 3-kinase) and phosphorylation of PDK2 did not differ among the refed groups (Fig. 3). Similar results were observed for IRS-2 phosphorylation and IRS-2 association with p85 (data not shown).

Insulin activation of PI 3-kinase and PKB leads to phosphorylation of multiple cellular proteins, including glycogen synthase kinase-3 (GSK-3). Phosphorylation of GSK-3 renders it inactive, and recent work has demonstrated that selective inhibition of GSK-3 activity reduces the expression of G6Pase mRNA (41). Serine phosphorylation of GSK-3 in rats refeed SU was not different from that in FD rats, and both were lower than in rats refeed ST or HF (Fig. 4). The amount of GSK-3 protein did not differ among the experimental groups (data not shown).

**Sucrose ingestion alters phosphorylation of CREB.** CREB is a putative target for GSK-3 and mediates cAMP-stimulation of G6Pase gene expression (42). Serine phosphorylation of CREB in rats refeed SU was not different from that in FD rats and both were greater than in rats refeed ST or HF (Fig. 5).

**DISCUSSION**

The present study examined postprandial regulation of G6Pase mRNA and activity. Ingestion of meals enriched in cornstarch or corn oil lowered G6Pase mRNA and activity compared with FD rats. In contrast, ingestion of a sucrose-enriched meal increased G6Pase mRNA compared with both the food-deprived and the other refeed groups and increased G6Pase activity compared with the other refeed groups.

Insulin suppresses G6Pase mRNA, and this effect appears to
override stimulatory effects of glucose, cAMP and dexamethasone on G6Pase mRNA expression (28,29,43). The dominant, suppressive effects of insulin on G6Pase mRNA predict that the postprandial environment (i.e., increased nutrient concentrations in the presence of hyperinsulinemia) would lower the levels of G6Pase mRNA relative to those in food-deprived rats. This occurred in rats refed the ST and HF diets but not in those refed the SU diet, where the mRNA level was increased. Insulin and glucose concentrations in the portal vein, and activation of proximal insulin signaling steps in the liver were not different among the refed groups at the 3-h time point. Thus, ingestion of the sucrose meal appeared not only to overcome the dominant, repressive effects of insulin on G6Pase gene expression but also to produce unique signals that increased G6Pase mRNA above FD levels.

Sucrose ingestion produced an intrahepatic environment characterized by a fourfold accumulation of X5P and 50% reduction in inorganic phosphate relative to the other two refed groups. These data likely reflect the increased removal of carbohydrate by the liver when fructose is present in the portal vein (7,8), in that accumulation of pentose pathway intermediates (e.g., X5P) occurs in response to excessive carbohydrate.

**FIGURE 1** mRNA levels of glucose-6-phosphatase (G6Pase; upper panel), glucose-6-phosphate translocase (G6PT; middle panel), and phosphoenolpyruvate carboxykinase (PEPCK; lower panel) in food-deprived rats (FD) and rats refed the starch (ST), sucrose (SU) or corn oil (HF) diets. Data are the ratio of the target mRNA to 18S mRNA. The top portion of each figure is a gel representative of n = 3/group. Values are means ± SEM, n = 6–8, with FD set to 1. Bars without a common letter differ, P < 0.05.

**FIGURE 2** Glucose-6-phosphatase (G6Pase) activity in whole liver homogenates from food-deprived rats (FD) and rats refed the starch (ST), sucrose (SU) or corn oil (HF) diets. Values are means ± SEM, n = 6–8. Bars without a common letter differ, P < 0.05.

**FIGURE 3** Tyrosine phosphorylation of IRS-1 (IRS1-pY), association of IRS-1 with the p85 subunit of phosphatidylinositol 3-kinase (PI 3)-kinase (IRS1-p85), and phospho-protein kinase B (p-PKB) in livers from food-deprived rats (FD) and rats refed the starch (ST), sucrose (SU) or corn oil (HF) diets. Liver supernatants were immunoprecipitated with an antibody against IRS-1 and then immunoblotted with either an anti-phosphotyrosine (pY) antibody or an antibody to the p85 subunit of PI 3-kinase as described in Materials and Methods. P-PKB was analyzed by immunoblot analysis as described in methods. The top portion of the figure is a gel representative of n = 2/group. Values are means ± SEM, n = 6–8, with FD set to 1.
availability (24,26) and sequestration of phosphate would be expected when phosphorylated intermediates are increased. It is possible that an intermediate such as X5P, which was selectively increased after sucrose ingestion, contributes to the unique changes in G6Pase observed in the present study (22,24,43–45).

In the present study we identified two potential mediators of the sucrose-induced upregulation of G6Pase mRNA, GSK-3 and CREB. A previous study demonstrated that the selective reduction of GSK-3 activity (as would occur when phosphorylated) reduced the expression of G6Pase in hepatoma cells (41). In the present study, serine phosphorylation of glycogen synthase kinase was low after food deprivation and sucrose ingestion, and high after ingestion of the starch- or corn oil–enriched meals. The nuclear factor CREB interacts specifically with the cAMP response element in genes controlled by the cAMP-mediated pathways of signal transduction (42). Included in this set of genes are G6Pase and PEPCK. The cAMP-dependent protein kinase phosphorylates CREB at a single serine residue, Ser133, and creates a sequence motif that is a consensus site for GSK-3 (42). Hierarchical phosphorylation of CREB at these two sites appears to be necessary for the full activation of transcription by CREB (42). In the present study, phosphorylation of Ser133 was reduced after ingestion of the starch- or corn oil–enriched meals, but was maintained at a level equivalent to food-deprived controls after sucrose ingestion. This outcome and the data pertaining to GSK-3 allow us to hypothesize that the unique intrahepatic environment created by ingestion of a sucrose-enriched meal may promote the transcriptional activation of CREB.

It is important to note that the above scenario, although consistent with an impairment in the suppression of G6Pase mRNA, does not necessarily explain the increase in G6Pase mRNA above levels in food-deprived rats. Clearly, unique signals are being generated in the liver after sucrose ingestion perhaps to prepare the liver to export excess phosphorylated intermediates.

Minassian et al. (46) previously reported that G6Pase activity was decreased in liver homogenates from rats refed a nonpurified, high carbohydrate meal. This inhibition was not observed in isolated microsomes. The present study confirms these observations in the context of two purified meals, one enriched in cornstarch and the other enriched in corn oil and extends those results by demonstrating a lack of suppression of G6Pase activity in liver homogenates after ingestion of a meal enriched in sucrose. This differential effect of sucrose ingestion cannot be attributed to selective alterations in the amount of catalytic subunit or G6PT proteins because refeeding did not affect either of these proteins. It should be noted that catalytic subunit and G6PT protein levels were increased when a sucrose-enriched diet was continuously provided for 1 wk (9). In addition, differences in the release of mannose-6-phosphatase activity, typically interpreted to reflect changes in the conformation of the enzyme (37), do not appear to explain the lack of suppression of G6Pase activity after ingestion of the sucrose-enriched meal. Several compounds have been shown to inhibit the activity of G6Pase including α-ketoglutarate, a proline metabolite, phosphoinositides, unsaturated fatty acids and unsaturated fatty acids bound to the glycogen granule (47,48). Sucrose ingestion, via metabolism of its monosaccharides, fructose and glucose (7), stimulates lipogenesis and the production of saturated fatty acids as well as glycogenesis. We therefore hypothesize that the inability to reduce G6Pase after ingestion of the sucrose meal may be due in part to the composition of fatty acids within the cell, associated with the endoplasmic reticulum membrane and/or associated with glycogen.

In conclusion, the present study demonstrates that the hepatic response to meal ingestion can be modulated by nutrient composition. Specifically, the intrahepatic environment created by the ingestion of sucrose paradoxically increases G6Pase mRNA above food-deprived levels without suppression of G6Pase activity. These changes are accompanied by unique alterations in the phosphorylation state of GSK-3 and CREB that may be relevant to the postprandial regulation of glycogen metabolism.

FIGURE 4 Phospho-glycogen synthase kinase (GSK)-3 α and β in livers from food-deprived rats (FD) and rats refed the starch (ST), sucrose (SU) or corn oil (HF) diets. Liver supernatants were immunoblotted as described in Materials and Methods. The top portion of the figure is a gel representative of n = 3/group. Values are means ± SEM, n = 6–8, with FD set to 1. Bars without a common letter differ, P < 0.05.

FIGURE 5 Phosphorylated CREB in lysates from food-deprived rats (FD) and rats refed the starch (ST), sucrose (SU) or corn oil (HF) diets. Liver supernatants were immunoblotted as described in Materials and Methods. The top portion of the figure is a gel representative of n = 2 (HF, lanes 10–11) or 3/group (FD, lanes 1–3; ST, lanes 4–6; SU, lanes 7–9). Values are means ± SEM, n = 6–8, with FD set to 1. Bars without a common letter differ, P < 0.05.
G6Pase. Future work will investigate the paradoxical regulation of G6Pase after sucrose ingestion by investigating the regulation of the G6Pase promoter by simple sugars, nutrient regulation of protein kinase A and the cAMP-mediated pathway, and the role of GS-3 and CREB in this regulation.

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LITERATURE CITED