A high-sucrose diet increases gluconeogenic capacity in isolated periportal and perivenous rat hepatocytes

MICHAEL E. BIZEAU, JEFFREY S. THRESHER, AND MICHAEL J. PAGLIASSOTTI
Exercise Science Research Institute, Arizona State University, Tempe, Arizona

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Bizeau, Michael E., Jeffrey S. Thresher, and Michael J. Pagliassotti. A high-sucrose diet increases gluconeogenic capacity in isolated periportal and perivenous rat hepatocytes. Am J Physiol Endocrinol Metab 280: E695–E702, 2001.—A high-sucrose (SU) diet increases gluconeogenesis (GNG) in the liver. The present study was conducted to determine the contribution of periportal (PP) and perivenous (PV) cell populations to this SU-induced increase in GNG. Male Sprague-Dawley rats were fed an SU (68% sucrose) or starch (ST, 68% starch) diet for 1 wk, and hepatocytes were isolated from the PP or PV region of the liver acinus. Hepatocytes were incubated for 1 h in the presence of various gluconeogenic substrates, and glucose release into the medium was used to estimate GNG. When incubated in the presence of 5 mM lactate, which enters GNG at the level of pyruvate, glucose release (nmol h⁻¹ mg⁻¹) was significantly increased by the SU diet in both PP (84.8 ± 3.4 vs. 70.4 ± 2.6) and PV (64.3 ± 2.5 vs. 38.2 ± 2.1) cells. Addition of palmitate (0.5 mM) increased glucose release from lactate in PP cells by 11.6 ± 0.5 and 20.6 ± 1.5% and in PV cells by 11.0 ± 4.4 and 51.1 ± 9.1% in SU and ST, respectively. When cells were incubated with 5 mM dihydroxyacetone (DHA), which enters GNG at the triosephosphate level, glucose release was significantly increased by the SU diet in both cell types. In contrast, glucose release from fructose (0.5 mM) was significantly increased by the SU diet in PV cells only. These changes in glucose release were accompanied by significant increases in the maximal specific activities of glucose-6-phosphatase (G-6-Pase) and phosphoenolpyruvate carboxykinase (PEPCK) in both PP and PV cells. Addition of lactate (0.5 mM) increased glucose release from lactate in PP cells by 6.9 ± 2.0 and 2.1) cells. Addition of palmitate (0.5 mM) increased glucose release from lactate in PP cells by 11.6 ± 0.5 and 20.6 ± 1.5% and in PV cells by 11.0 ± 4.4 and 51.1 ± 9.1% in SU and ST, respectively. When cells were incubated with 5 mM dihydroxyacetone (DHA), which enters GNG at the triosephosphate level, glucose release was significantly increased by the SU diet in both cell types. In contrast, glucose release from fructose (0.5 mM) was significantly increased by the SU diet in PV cells only. These changes in glucose release were accompanied by significant increases in the maximal specific activities of glucose-6-phosphatase (G-6-Pase) and phosphoenolpyruvate carboxykinase (PEPCK) in both PP and PV cells. These data suggest that the SU diet influences GNG in both PP and PV cell populations. It appears that SU feeding produces changes in GNG via alterations in at least two critical enzymes, G-6-Pase and PEPCK.

liver; diet composition; glucose production

Type 2 diabetes is characterized by an increase in the contribution of gluconeogenesis (GNG) to glucose production (30). Dietary nutrients may contribute to changes in the contribution of GNG to glucose production. In rats, 1 wk of high-sucrose (SU) diet feeding increased the capacity for GNG in isolated hepatocytes (25) and perfused livers (22) and the contribution of GNG to glucose appearance in vivo (unpublished observations). The sugar-induced increase in gluconeogenic capacity from precursors that pass through pyruvate (e.g., lactate, alanine) appears to be due, in part, to an increase in the activity of phosphoenolpyruvate carboxykinase (PEPCK) (21, 24). SU feeding also increased glucose release from dihydroxyacetone (DHA), which enters GNG above PEPCK (22). One aim of the present study was to determine the site of adaptation that contributes to the SU-induced increase in GNG from DHA.

Metabolism within the liver acinus has been classified or separated with respect to blood flow along the sinusoid. It is generally accepted, on the basis of functional (18) and enzymatic (2) measurements, that periportal (PP) regions of the acinus have a greater capacity for GNG compared with perivenous (PV) regions. For example, the maximal activities of PEPCK and glucose-6-phosphatase (G-6-Pase) are between 1.8 and 2.0 times greater in the PP vs. the PV region of the acinus (11). In addition, the partitioning of pyruvate into pyruvate carboxylase, and thus GNG, was increased about threefold in PP compared with PV cells isolated from starved rats (10).

The contribution of PP and PV cells to GNG depends in part on the microenvironment (e.g., blood flow, hormone concentrations) (11). Dietary composition also appears to modify gluconeogenic enzyme activity. Wimmer et al. (36) demonstrated that high-fat and high-protein diets increased PEPCK activity to a greater extent in PV compared with PP cells. Thus one adaptation to changes in dietary nutrient composition may be to reduce metabolic heterogeneity across the liver acinus. Because it has been postulated that hepatocyte heterogeneity promotes energy conservation and metabolic control by combining complementary pathways and separating antagonistic pathways (11), it would be predicted that a reduction in heterogeneity would alter the regulation of both energy metabolism and hepatic glucose production. Therefore, the second aim of this study was to determine whether sucrose-induced changes in GNG are localized to specific liver cell populations.

Fatty acid supply to the liver plays an important role in determining both the ability of insulin to suppress glucose production (28) and the rate of GNG (8). How-

Address for reprint requests and other correspondence: M. E. Bizeau, Univ. of Colorado Health Sciences Center, Division of Endocrinology, Metabolism and Diabetes, Campus Box B151, 4200 E Ninth Ave., Denver, CO 80262 (E-mail: Michael.Bizeau@UCHSC.edu).

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ever, in previous studies we have not observed significant changes in adipose tissue stores or circulating free fatty acids with sucrose feeding (21–24). The third aim of this study was to determine whether sucrose-induced changes in GNG are accompanied by changes in the ability of fatty acids to stimulate GNG.

During absorption, sucrose is hydrolyzed to fructose and glucose. Several studies have demonstrated that the fructose moiety of the sucrose molecule plays a crucial role in the development of sucrose-induced insulin resistance (31–33). Fructose is largely metabolized by the liver (19), and the presence of fructose stimulates glucose phosphorylation (34, 35). Thus a sucrose-containing meal will result in a large, and perhaps excessive, carbohydrate uptake by the liver. It is presently unknown whether the chronic presentation of fructose to the liver alters fructose uptake or glucokinase (GK) activity. The final aim of the present study was to determine whether sucrose feeding altered fructose uptake, GNG from fructose, or GK activity in PP and PV cells.

METHODS

Materials. Collagenase, type I, was obtained from Worthington Biochemical (Lakewood, NJ). Glucose, fructose, and alanine aminotransferase assay kits and digitonin were from Sigma (St. Louis, MO). Gelatin was purchased from Difco (Detroit, MI). All other chemicals were of reagent grade and were obtained from Sigma.

Experimental animals and feeding protocol. Male Sprague-Dawley rats were obtained from an institutional breeding stock weighing ~180 g. Animals were housed individually in a temperature-controlled room with a 12:12-h light-dark cycle and free access to food and water. All procedures for animal use were approved by the Institutional Animal Care and Use Committee at Arizona State University. Upon initiation of the study, all animals were provided free access to a semipurified high-starch (ST) diet (% of total calories: 68 cornstarch, 20 protein, 12 fat) for a 2-wk baseline period. Food intake was measured daily and body weight recorded weekly. After the 2-wk baseline period, rats were either switched to an SU (% of total calories: 68 sucrose, 20 protein, 12 fat) diet or remained on the ST diet for 1 wk. During this week, rats were fed 95% of the average food intake recorded during the 2nd week of baseline feeding. Feeding 95% of baseline calories during the experimental feeding period results in rats with similar rates of weight gain and body composition (24). Complete diet composition is presented in Table 1. Rats were between 2.5 and 3 mo of age at the time of study.

Hepatocyte isolation. PP and PV hepatocytes were isolated from 24-h-fasted rats (to deplete liver glycogen stores) following the basic procedures developed by Lindros and Penttila (14) and Quistorff (27) as modified by Jones and Titheradge (10). For preparation of PV-enriched hepatocytes, rats were anesthetized with an intramuscular injection of ketamine (50 mg/kg), xylazine (10 mg/kg), and acepromazine (5 mg/kg). The abdominal cavity was opened, and the portal vein and superior vena cava were cannulated. The liver was perfused in the anterograde direction with calcium-free Krebs-Ringer bicarbonate buffer equilibrated with 95% O2-5% CO2 at 37°C and pH 7.4. Once the liver was cleared of blood, ~50 ml of the initial perfusate were allowed to drain to waste. Digitonin (2 ml of 10 mg/ml in Krebs-Ringer and 20 mM HEPES, pH 7.4) was then injected into the portal vein until the reticular pattern described by Lindros and Penttila was observed. Flow was rapidly switched to the retrograde direction to remove digitonin and continued at ~45 ml/min until 50 ml of perfusate were collected. For PP hepatocytes, digitonin (2.5 ml) was injected via the superior vena cava until the characteristic dot pattern of PV destruction was observed (14). Digitonin was removed by perfusing in the anterograde direction at ~45 ml/min until 50 ml of perfusate were collected. After digitonin washout, hepatocytes were prepared using standard collagenase perfusion methods (4).

Glucose release from lactate and DHA. Before use in incubations, hepatocytes were suspended at a final concentration of 30 mg wet wt/ml in Krebs-Ringer bicarbonate buffer containing 1% gelatin and equilibrated for 25 min with 95% O2-5% CO2 at 37°C. Cell suspensions (2 ml) were incubated (in triplicate) for 1 h in the presence of one of the following substrates or substrate combinations: 5 mM lactate, 5 mM DHA, or 5 mM lactate + 0.5 mM palmitate (bound to albumin). Incubations were terminated by addition of an equal amount of 0.6 M perchloric acid, with subsequent neutralization of the supernatant with 1 M KHCO3. Neutralized supernatants were stored at ~70°C.

Glucose release from fructose. Before use in incubations, cells were treated as described in Glucose release from lactate and DHA. Cell suspensions (3 ml) were incubated (in triplicate) for 1 h in the presence of 0.5 mM fructose. Samples were withdrawn at 10, 20, 30, 40, 50, and 60 min for determination of fructose disappearance and glucose release. Sample processing was performed as described in Glucose release from lactate and DHA.

Enzyme analysis. G-6-Pase activity was measured in freeze-thawed cell suspensions containing ~30 mg cells/ml at the following concentrations of G-6-P (in mM: 0.5, 1, 2.5, 5, and 10) according to the methods of Burchell et al. (5). Nonspecific phosphatase activity was estimated using p-nitrophenyl phosphate, as described previously (20). PEPCK activity was determined on aliquots of cell suspensions (~60 mg cells/ml) that had been frozen in a medium containing 0.2 M sucrose, 50 mM HEPES, and 1.5 mM dithiothreitol, pH 7.5. The aliquots were thawed and centrifuged at 15,300 g for 30 min. The supernatant was then assayed for PEPCK activity as described by Bentle and Lardy (3). Alanine aminotransferase (AAT, Sigma kit) and glutamate dehydrogenase (GDH) (16) were measured spectrophotometrically in freeze-thawed cell suspensions pretreated with 0.1% Triton X-100. Glucokinase activity was determined on aliquots of cell sus-
pensions (~60 mg cells/ml) that had been frozen in a medium containing 150 mM KCl, 50 mM HEPES, and 1.5 mg/ml dithiothreitol, pH 7.5. The aliquots were thawed and centrifuged at 20,300 g for 30 min. The supernatant was then assayed for glucokinase activity as described by Davidson and Arion (7).

**Metabolite assays.** Glucose and fructose were measured using assay kits (Sigma) according to the suppliers’ instructions, with modifications. Briefly, fructose was assayed fluorometrically after removal of glucose from the incubation medium that used glucose oxidase and catalase. Protein was determined by the method of Lowry et al. (15).

**Cell culture.** Hepatocytes were obtained by collagenase perfusion of the liver from male Sprague-Dawley rats weighing ~300 g that had been fed the ST diet for 3 wk. Cell viability was determined by trypan blue exclusion (>90%). Cells were plated at a density of 3.5 × 10^6 cells/60 mm in a Primaria culture dish (Falcon, Becton-Dickinson, Lincoln Park, NJ) in Williams E media (Life Technologies) modified by lack of glucose and methyl linoleate and supplemented with 10% fetal bovine serum (FBS), 23 mM HEPES (pH 7.4), 26 mM sodium bicarbonate, 2 mM L-glutamine, 2 nM dexethasone, 1 μM insulin, 11 mM glucose, 100 μg/ml penicillin, and 100 μg/ml streptomycin. After a 4-h to 6-h attachment period, cells were incubated with this medium [lacking FBS and containing either 5.5 mM glucose (LG, n = 4 rats), 0.5 mM fructose + 5.0 mM glucose (F, n = 4 rats), or 15 mM glucose (HG, n = 4 rats)]. Medium was changed every 4 h to ensure that fructose was never depleted. After 24 h, cells were harvested and RNA was isolated.

**RNA extraction and Northern blot analysis.** Total RNA was prepared from 3–5 pooled culture dishes using TRIzol (GIBCO BRL, Baltimore, MD) and following the manufacturer’s protocol. For Northern analysis, 20 μg of RNA were resolved on 1% agarose-formaldehyde gels and transferred onto membranes by capillary elution. Membranes were ultraviolet-crosslinked at 254 nm. Membranes were hybridized, washed, and exposed to X-ray film at −80°C, and autoradiographs were quantified using a laser densitometer. Membranes were stripped and reprobed as specified by the manufacturer. The PEPCK cDNA was released from the plasmid pCK10 by PstI digestion (37). The cDNA for the catalytic subunit of G-6-Pase was released from BS/G-6-Pase/7.1 by HindIII/BamHI digestion (13). The cDNA insert for ribosomal phosphoprotein PO (RPP0), used as a control, was excised from p36B4 by PstI digestion (12). The cDNA probes were prepared by the random priming method as specified by the manufacturer’s instructions (Boehringer Mannheim).

**Data analysis and statistical procedures.** Data from each experiment were represented as the average value of each triplicate incubation. The rates of fructose uptake and glucose release from fructose were calculated from the initial 10-min time point of the incubation, because experiments indicated that by 20 min most of the fructose in the incubation medium was metabolized. Data were analyzed using one-way ANOVA and repeated-measures ANOVA where appropriate. If the overall F was significant, comparisons between mean values were made using a Student-Newman-Keuls test. Significance was set at P < 0.05 for all comparisons. All data are presented as means ± SE.

### RESULTS

**General animal and cell characteristics.** After 1 wk on the respective diets, there were no differences in body weight between diet groups (SU = 321 ± 5.7 g, n = 8; ST = 326 ± 4.8 g, n = 8). As has been previously demonstrated with the digitonin perfusion method (6, 10), hepatocytes isolated from the ST region contained greater AAT activity, whereas PV hepatocytes contained greater GDH activity (Table 2). There were no effects of the diet on either AAT or GDH maximal activities in PP and PV cell populations. Total cell yield per liver was greater from PP compared with PV hepatocyte isolations (Table 2). Initial liver glycogen concentration was not different between groups or cell populations and was <2 μg/mg cell wet weight.

**Glucose release from lactate and DHA.** When isolated PP hepatocytes from 24-h-fasted rats were incubated in the presence of 5 mM lactate or 5 mM DHA, the rate of glucose release into the medium was greater in hepatocytes obtained from SU-fed animals than in those fed a ST diet. A similar response was observed in PP hepatocytes (Table 3). In PP and PV cells, addition of palmitate increased glucose production from lactate to a greater degree in hepatocytes from ST compared with SU animals (Fig. 1).

**Glucose release from fructose.** Fructose disappearance from the medium was greater in PV vs. PP cells. There was no diet effect on fructose disappearance

### Table 3. Glucose release in isolated PP and PV hepatocytes

<table>
<thead>
<tr>
<th>Glucose Source</th>
<th>PP</th>
<th>PV</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 mM Lactate</td>
<td>70.4 ± 2.6*</td>
<td>38.2 ± 2.1</td>
</tr>
<tr>
<td>5 mM DHA</td>
<td>127.7 ± 6.2</td>
<td>117.6 ± 4.4</td>
</tr>
<tr>
<td>5 mM Fructose</td>
<td>84.8 ± 3.4**</td>
<td>64.3 ± 2.5**</td>
</tr>
</tbody>
</table>

*PP > PV (P < 0.05); †SU > ST (P < 0.05).

Isolated PP and PV hepatocytes were incubated in triplicate as described in METHODS, with the substrate concentrations of lactate and dihydroxyacetone (DHA) for 1 h, and glucose release into the medium was measured. Data are means ± SE of 4 cell preparations/group for rate of glucose release in mmol·mg^-1·h^-1. *PP > PV (P < 0.05); †SU > ST (P < 0.05).

### Table 2. Marker enzymes and cell yields obtained during PP and PV cell isolations from ST- and SU-fed rats

<table>
<thead>
<tr>
<th>Enzyme Activity</th>
<th>PP Starch</th>
<th>PV Starch</th>
<th>PP Sucrose</th>
<th>PV Sucrose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine aminotransferase</td>
<td>113.5 ± 4.2*</td>
<td>60.1 ± 3.1</td>
<td>118.5 ± 4.8*</td>
<td>65.3 ± 3.5</td>
</tr>
<tr>
<td>Glutamate dehydrogenase</td>
<td>840.8 ± 47.2*</td>
<td>1,530.7 ± 70</td>
<td>857.6 ± 53.2*</td>
<td>1,568.3 ± 86.3</td>
</tr>
<tr>
<td>Cell yield</td>
<td>1.38 ± 0.11*</td>
<td>0.98 ± 0.08</td>
<td>1.43 ± 0.12*</td>
<td>0.96 ± 0.1</td>
</tr>
</tbody>
</table>

Enzyme data are means ± SE of duplicate assays for the rate of maximal specific activity in nmol·mg protein^-1·min^-1, and cell yield data are means ± SE in g of cells/liver, with 8 individual cell preparations/group. PP, periportal; PV, perivenous; ST, starch; SU, sucrose. *PP different from PP for a single diet group (P < 0.05).
Glucose release from fructose was significantly elevated only in the PV cells from the SU group (Table 4).

**Enzyme activities.** Because gluconeogenic capacity was increased in both PP and PV hepatocytes from the SU-fed animals, we wished to examine possible enzymatic steps in the gluconeogenic pathway that could contribute to this increase. In PP hepatocytes, G-6-Pase activity was significantly elevated in the SU-fed group compared with ST-fed animals at G-6-P concentrations >0.5 mM (Fig. 2A). In PV cells, G-6-Pase activity was significantly greater in cells from the SU- vs. ST-fed animals at all G-6-P concentrations examined (Fig. 2B). In both PP and PV cells, PEPCK specific activity was significantly elevated in the SU-fed group compared with the ST group (Fig. 3). Glucokinase activity in PP and PV cells was not significantly different between ST and SU (Fig. 4). Glucokinase activity was significantly higher in PV compared with PP cells (Fig. 4).

**Heterogeneity of gluconeogenesis across the liver acinus.** To assess the effects of a sucrose diet on the heterogeneity of GNG across the liver acinus, glucose release from lactate or DHA and gluconeogenic enzyme activity were compared as PP-to-PV ratios (PP/PV; Table 5). PP/PV for glucose release from lactate and PEPCK activity were significantly decreased in the SU group. SU did not have a significant effect on PP/PV for glucose release from DHA or the activity of G-6-Pase.

### Table 4. Glucose release from fructose and fructose uptake from 0.5 mM fructose in PP and PV hepatocytes

<table>
<thead>
<tr>
<th>Fructose uptake or Glucose release</th>
<th>Starch PP</th>
<th>Starch PV</th>
<th>Sucrose PP</th>
<th>Sucrose PV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fructose uptake</td>
<td>42.5 ± 6.8</td>
<td>56.2 ± 3.1*</td>
<td>44.8 ± 6.9</td>
<td>54.4 ± 4.9*</td>
</tr>
<tr>
<td>Glucose release</td>
<td>38.8 ± 2.8</td>
<td>40.5 ± 5.3</td>
<td>43.8 ± 4.5</td>
<td>53.2 ± 5.1†</td>
</tr>
</tbody>
</table>

Isolated PP and PV hepatocytes were incubated in triplicate, as described in METHODS, with the substrate fructose for 1 h, and glucose release into the medium was measured. Values are means ± SE of 4 cell preparations/group for rate of glucose release or fructose uptake in nmol/mg cell wet wt⁻¹ h⁻¹, based on measurements obtained during the first 10 min of the incubation. *PV > PP (P < 0.05); †SU > ST (P < 0.05).
Cell culture experiments. To examine whether the sucrose-induced increase in PEPCK and G-6-Pase might result from fructose/glucose-mediated changes in gene expression, cell culture experiments were performed. Incubation of primary hepatocytes with either 0.5 mM fructose + 5 mM glucose or 15 mM glucose increased G-6-Pase mRNA 7- to 10-fold compared with incubations with 5.5 mM glucose (Fig. 5). These incubations did not increase PEPCK mRNA.

DISCUSSION

General animal and cell characteristics. The goal of the present study was to examine the effect of an SU diet on gluconeogenic capacity in hepatocytes isolated from the PP and PV regions of the liver acinus. To perform this, hepatocytes were isolated using a modification of the digitonin perfusion method. Cells obtained by this method demonstrated a pattern of marker enzyme activity typically associated with PP and PV cell preparations isolated by this procedure (6, 10). This pattern was characterized by increased AAT activity in cells isolated from the PP region and increased GDH activity in cells isolated from the PV region. Thus cell preparations designated as PP were enriched with PP cells and those designated PV were in fact enriched with PV cells. Functional measurements of gluconeogenic capacity were performed on 24-h-fasted animals to ensure that liver glycogen would be depleted and not contribute to estimates of GNG. Initial liver glycogen concentration was <2 µg/mg; therefore, glucose release in these experiments primarily represented glucose formed from GNG.

Characteristics of glucose release. Given the fact that an SU diet can increase GNG (22) and diet composition can alter the distribution of gluconeogenic enzymes across the liver acinus (36), it was of interest to determine whether the SU-induced increase in GNG was preferentially localized to a specific region of the liver acinus. When isolated hepatocytes were incubated with 5 mM lactate or 5 mM DHA, both PP and PV cells from the SU-fed animals displayed greater rates of glucose release compared with ST-fed controls. Thus the SU diet increased GNG from lactate and DHA in both the PP and PV cell preparations. These data suggest that the sucrose-induced increase in GNG in-

![Graph](image)

**Fig. 3.** Phosphoenolpyruvate carboxykinase (PEPCK) specific activity in PP and PV hepatocytes from ST- and SU-fed animals. *ST significantly different (P < 0.5) from SU for a specific cell population. Values are means ± SE; n, 4–6/group for each cell type.

![Graph](image)

**Fig. 4.** Glucokinase kinetics in PP (A) and PV (B) hepatocytes from ST- and SU-fed animals. Freeze-thawed cell suspensions (30 mg/ml) were assayed for glucokinase activity as described in Methods. Values are means ± SE of no. of individual hepatocyte preparations given in parentheses.

### Table 5. PP/PV activity ratios of glucose release and enzyme activity

<table>
<thead>
<tr>
<th></th>
<th>PP/PV Ratio</th>
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<tbody>
<tr>
<td></td>
<td>ST</td>
</tr>
<tr>
<td>Glucose from 5 mM lactate</td>
<td>1.84 ± 0.15*</td>
</tr>
<tr>
<td>Maximal PEPCK activity</td>
<td>1.58 ± 0.13*</td>
</tr>
<tr>
<td>Glucose from 5 mM DHA</td>
<td>1.08 ± 0.18</td>
</tr>
<tr>
<td>Maximal G-6-Pase Activity</td>
<td>1.52 ± 0.17</td>
</tr>
</tbody>
</table>

Values are means ± SE for 4–6 hepatocyte preparations per cell population per diet group. PEPCK, phosphoenolpyruvate carboxykinase; G-6-Pase, glucose-6-phosphatase. *SU significantly different from ST (P < 0.05).
Thus the lack of an SU-induced increase in GNG from percentage was increased to fructose uptake in PV cells from the ST group, and this was set at 1.0.

In contrast, glucose release represented a concomitant increase in fructose uptake, which did not occur from fructose could not increase further without a flow) regions of the liver acinus.

Fructose uptake and glucose release. Fructose appears to be the primary mediator of sucrose-induced insulin resistance and accelerated GNG (31–33). Therefore, it was of interest to determine whether the SU diet increased the capacity for fructose uptake and/or GNG from fructose. Results from this study indicate that fructose uptake was not increased by the SU diet in either cell population. However, the SU diet increased GNG from fructose in PV but not PP cells. Although this result may appear to be in conflict with the SU-induced increase in GNG from DHA, further evaluation suggests otherwise. In the ST group, glucose release represented ~91% of fructose uptake in PP cells. Therefore, under the conditions imposed in these experiments (0.5 mM fructose), glucose release from fructose could not increase further without a concomitant increase in fructose uptake, which did not occur. In contrast, glucose release represented ~72% of fructose uptake in PV cells from the ST group, and this percentage was increased to ~98% after SU feeding. Thus the lack of an SU-induced increase in GNG from fructose in PP cells may be due both to their lower capacity for fructose uptake (relative to PV cells) and to the low (but physiological) fructose concentrations used. It should be noted that the choice of 0.5 mM fructose was not arbitrary. Previous studies have reported that concentrations >0.5 mM can reduce ATP levels in the liver (19). Furthermore, maximal fructose-mediated stimulation of glucose phosphorylation appears to occur between 0.2 and 0.5 mM fructose (35).

Palmitate stimulation of glucose release from lactate. The above experiments were performed with a medium devoid of fatty acids and thus suggest that the adaptation to sucrose feeding that results in an increased capacity for GNG resides within the liver. However, accelerated GNG requires energy, and the extent of hepatic fatty acid oxidation is closely linked to the rate of GNG (8). Delivery of free fatty acids to the liver is not increased by SU feeding (24); therefore, the present study compared the ability of palmitate to stimulate lactate GNG between the two diet groups to assess whether this might be a component of the SU-induced adaptation in GNG. The data suggest that palmitate stimulated GNG from lactate to a greater degree in hepatocytes from the ST group compared with the SU group. Thus it may be that increased endogenous lipid stores associated with SU feeding (25) support accelerated rates of GNG after sucrose feeding. In support of this hypothesis, we have recently demonstrated that inhibition of endogenous fat oxidation reduced GNG from lactate in hepatocytes isolated from sucrose- but not starch-fed rats (unpublished observations).

Sites that contribute to increased GNG from lactate and DHA. Previous studies demonstrated that maximal hepatic PEPCK activity was increased after sucrose feeding (21, 24). Clearly, this adaptation contributes to the increased glucose release from lactate. The present study extends these previous observations by demonstrating that the SU-induced increase in PEPCK occurred in both PP and PV cell populations. In addition to PEPCK, G-6-Pase activity was increased in PP and PV hepatocytes from SU-fed animals. This adaptation would contribute to the SU-induced increase in GNG from lactate and DHA. It should be noted that changes in G-6-Pase activity were not due to effects on nonspecific phosphatases, because diet effects were not observed when p-nitrophenyl phosphate was used as substrate (data not shown).

Previous studies have suggested that glucose (1, 17) and metabolites of hepatic glucose metabolism (1) can stimulate G-6-Pase gene expression. Thus alterations in glucose uptake/metabolism may provide a mechanistic link between sucrose feeding and changes in G-6-Pase activity, because fructose stimulates glucose phosphorylation in liver (34, 35). Results from the present study demonstrate that fructose increased G-6-Pase mRNA in the presence of physiological concentrations of glucose. The increase in mRNA under this condition was only slightly less than that which occurred in the presence of 15 mM glucose. These data imply that the increase in G-6-Pase mRNA after exposure to fructose and physiological glucose concentra-
tions resulted from fructose-mediated changes in glucose metabolism. This hypothesis is presently under investigation.

In contrast to G-6-Pase, fructose or glucose did not increase the expression of PEPCK. This result was not unexpected, as recent studies have indicated that glucose can downregulate PEPCK gene expression (29). It is likely that sucrose-induced changes in PEPCK activity result from impaired or altered hormone action (9, 26).

**GK and sucrose feeding.** After 1 wk of sucrose feeding, basal rates of glucose production are not increased in vivo (21, 23, 24). This implies that additional adaptations/responses compensate for the sucrose-mediated increase in G-6-Pase. In vivo, hyperinsulinemia could help sustain normal rates of glucose production after sucrose feeding. However, previous studies have not observed a significant elevation in basal insulin concentrations after 1 wk of sucrose feeding (23). Fructose stimulates the activity of GK (34, 35), and an elevation in GK activity could help to sustain normal rates of glucose production despite elevated G-6-Pase. In the present study, GK activity was measured on cell suspensions obtained from 24-h-fasted rats before incubations. Under these conditions, GK activity in PP and PV cells was not significantly different between ST and SU groups. These data suggest that sucrose feeding does not produce a chronic change in GK activity. To the extent that these results can be generalized to the in vivo condition, they also suggest that changes in GK activity may not compensate for the elevation in G-6-Pase activity after sucrose feeding. Additional studies are under way to investigate GK activity in the fed state, GK translocation, and GK regulation by its regulatory protein.

**SU diet and liver cell heterogeneity.** Although SU feeding increased GNG in PP and PV cell populations, the magnitude of change was greater in PV cells. When glucose release from lactate was examined as PP/PV, a greater adaptation was observed in PV cells. In ST-fed animals, PP/PV was ~1.8, whereas in the SU group it was ~1.3. A similar shift in PP/PV activity for PEPCK was also observed (1.5 in ST and 1.2 in SU). Wimmer et al. (36) demonstrated that a single week of high-fat feeding resulted in an overall increase in liver PEPCK activity that was greater in the PV region compared with the PP region. It would appear that diet composition can reduce the heterogeneity of GNG across the liver acinus. However, this effect appears to involve GNG only from precursors that pass through pyruvate. Sucrose feeding increases reliance on GNG for glucose production and also increases gluconeogenic capacity in PV cells (i.e., reduces heterogeneity). This loss of heterogeneity in gluconeogenic capacity may contribute to the early appearance (1 wk) of hepatic insulin resistance in rats fed a high-sucrose diet (23). Normal zonation of carbohydrate metabolism in the liver predicts that PV cells serve as buffers of glucose release from PP cells. Reductions in this zonation, like those demonstrated in the present study, suggest that additional adaptations (within or outside of the liver) would be required to compensate for this loss of buffering.

Fructose uptake and the capacity for glucose uptake (based on GK activity) was greater in PV compared with PP cells. The greater relative change in GNG from lactate and PEPCK in PV cells could therefore be related to the extent of carbohydrate uptake, the metabolic fate of carbohydrate, or the lower rate of GNG in this cell population.

**Summary.** Sucrose feeding increased GNG from precursors that enter the pathway at both pyruvate and the triosephosphate levels. The increase in GNG that follows SU feeding occurs to a greater degree in the PV compared with the PP region for substrates that pass through pyruvate. The increased capacity for GNG after SU feeding is, in part, due to the upregulation of two key gluconeogenic enzymes, PEPCK and G-6-Pase. Overall, it would appear that the composition and amount of energy provided in the diet can influence hepatic GNG and liver cell heterogeneity. Therefore, dietary nutrients may make a significant contribution to both the enzymatic profile and the metabolic capacity of liver cells.

We speculate that sucrose-induced changes in G-6-Pase activity result from fructose-mediated changes in hepatic glucose metabolism and that endogenous lipid stores provide oxidative support for accelerated rates of GNG after sucrose feeding.

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