Sucrose diets increase glucose-6-phosphatase and glucose release and decrease glucokinase in hepatocytes

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Sucrose diets increase glucose-6-phosphatase and glucose release and decrease glucokinase in hepatocytes. J Appl Physiol 91: 2041–2046, 2001.—A high-sucrose diet (SU) decreases insulin action in the liver (Pagliassotti MJ, Shahrokhi KA, and Moscarello M. Am J Physiol Regulatory Integrative Comp Physiol 266: R1637–R1644, 1994). The present study was conducted to characterize the effect of SU on glucagon action in isolated periporal (PP) and perivenous (PV) hepatocytes by measuring glucagon-stimulated glycolysis and glucose release. Male rats were fed a SU (68% sucrose) or starch diet (ST, 68% starch) for 1 wk, and hepatocytes were isolated from PP or PV regions (n = 4/diet/cell population). Hepatocytes were incubated for 1 h in the presence of varying concentrations of glucagon (0–100 nM). In PP and PV cells, glucagon stimulation of glucose release and glycogenolysis (sum of glucose release and lactate accumulation) was not significantly different between SU and ST cells. However, in the SU PP cells, glucose release was increased compared with ST PP cells, both in the absence of glucagon (76.1 ± 4 vs. 54.8 ± 3 nmol·h⁻¹·mg cell wet wt⁻¹) and at all glucagon concentrations. In SU-fed PV cells, glucose release was increased compared with ST PV cells in the absence of glucagon (79.3 ± 5 vs. 56.4 ± 5 nmol·h⁻¹·mg cell wet wt⁻¹) and at low glucagon concentrations. Maximal glucose-6-phosphatase activity (in nmol·min⁻¹·mg protein⁻¹) was elevated in SU compared with ST cells (61.4 ± 3 vs. 37.5 ± 4 in PP and 37.5 ± 4 vs. 29.5 ± 3 in PV cells). In contrast, maximal glucokinase activity (in nmol·min⁻¹·mg protein⁻¹) was elevated in ST compared with SU cells (15.9 ± 2 vs. 12.1 ± 1 in PP and 19.4 ± 2 vs. 14.2 ± 1 in PV cells). These data demonstrate that SU increases the capacity for glucose release in both PP and PV hepatocytes, in part because of reciprocal changes in glucose-6-phosphatase and glucokinase.

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Dietary nutrients can rapidly and directly influence hepatic metabolism. For example, in rats, 1 wk of high-sucrose diet (SU) feeding decreases the ability of insulin to suppress hepatic gluconeogenesis in vitro (19) and glucose production in vivo (19, 21) and increases the capacity for gluconeogenesis in both isolated hepatocytes (22) and perfused livers (20). After 1 wk of SU feeding, insulin levels and basal glucose production are not increased (19, 21). Thus, despite insulin resistance and an increased capacity for hepatic gluconeogenesis, the liver maintains appropriate rates of basal glucose production. Hepatic adaptations in this dietary model can be used to further the understanding of environmental factors that contribute to the development of impaired glucose tolerance and Type 2 diabetes as well as to the autoregulation of hepatic glucose production.

Glucagon is an important regulator of hepatic glucose production, stimulating both glycolysis and gluconeogenesis (9). Although SU feeding does not significantly change circulating levels of glucagon, it is presently unknown whether glucagon action to stimulate glucose release or glycolysis in the liver is altered by this diet. Isolated hepatocytes are an ideal system to study glucagon action in the liver because in vivo experiments using glucagon infusions induce hyperglycemia that becomes a variable in itself that must be controlled for. The hepatic acinus, the functional unit of the liver, can be divided into periporal (PP) and perivenous (PV) regions with respect to blood flow (23). Within the acinus, glucagon receptors as well as the enzymes of carbohydrate metabolism are distributed in a heterogeneous pattern (12). The PP region of the acinus contains greater amounts of the glucagon receptor and glucagon receptor mRNA as well as gluconeogenic enzymes (14). Therefore, glucagon may exert a greater action in the PP region of the acinus, perhaps leading to greater glucose release in PP cells with glucagon stimulation. In addition, it has been demonstrated that PP and PV hepatocytes do not adapt homogeneously to dietary perturbations (4, 24), thus raising the following question: If a SU diet alters glucagon action, are both the PP and PV cell population similarly affected? Therefore, isolation and study of these two hepatocyte populations can provide valuable information as to how the liver adapts to dietary nutrients.

The glycogenolytic and gluconeogenic pathways both provide glucose-6-phosphate (G-6-P) that can be de-
phosphorylated by the enzyme glucose-6-phosphatase (G6Pase) to glucose. Glucose can be released from the hepatocyte or rephosphorylated by the enzyme glucokinase (GK) and retained within the hepatocyte. Thus glucose release from the hepatocyte is a function of the activities of GK and G-6-P. The present study was undertaken to examine the effects of SU on glucagon action and the enzymes involved in the terminal step of glucose release from the hepatocyte.

METHODS

**Experimental animals and feeding protocol.** Male Sprague-Dawley rats weighing ~180 g were obtained from an institutional breeding stock. All 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. On initiation of the study, all animals were provided free access to a semipurified high-starch diet (ST; % of total calories = 68 cornstarch, 20 protein, 12 fat) for a 2-wk baseline period. Food intake was measured daily, and body weight was recorded weekly. After the 2-wk baseline period, rats were switched to either SU (% of total calories = 68 sucrose, 20 protein, 12 fat) or remained on ST for 1 wk. During this week, rats were fed 95% of the average food intake recorded during the second week of baseline feeding. Feeding 95% of baseline calories during the experimental feeding period resulted in rats with similar rates of weight gain and body composition (21). Complete diet composition is presented in Table 1.

**Hepatocyte isolation.** PP and PV hepatocytes were isolated from rats fed normally the previous evening according to the basic procedures developed by Lindros and Penttila (15) as modified by Jones and Titheradge (11). 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 was allowed to drain to waste. Digitonin (2 ml of 10 mg/ml in Krebs-Ringer, 20 mM HEPES, pH 7.4) was then injected into the portal vein until the reticular pattern described by Lindros and Penttila (15) was observed. Flow was rapidly switched to the retrograde direction to remove digitonin and continued at ~45 ml/min until 50 ml of perfusate was 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 (15). Digitonin was removed by perfusing in the anterograde direction at ~45 ml/min until 50 ml of perfusate was collected. After digitonin washout, hepatocytes were prepared by using standard collagenase perfusion methods (2). The initial quality of the cell preparation was assessed by trypan blue exclusion (0.2% final concentration). Only preparations with >90% dye exclusion were used in cell incubations.

**Hepatocyte incubations.** Before use in incubations, hepatocytes were suspended at a final concentration of 30 mg wet weight/ml in Krebs-Ringer bicarbonate buffer containing 1% gelatin and equilibrated for 25 min with 95% O₂-5% CO₂ at 37°C. Cell suspensions (2.0 ml) were incubated for 1 h with or without glucagon (in mM: 0, 0.01, 0.1, 1, 10, and 100). For determination of glucose and lactate, 250 μl of the cell suspensions were removed before (0 min) and after (60 min) incubations and added to an equal amount of 0.6 M perchloric acid. Neutralization of the perchloric acid supernatants was performed by using 1 M KHCO₃. For glycogen determinations, a 250-μl aliquot of cell suspension was removed before (0 min) and after (60 min) the incubations and rapidly centrifuged to pellet the cells. The supernatant was discarded, and the cell pellet was frozen in liquid nitrogen and stored at -80°C until analysis.

**Enzyme analysis.** G6Pase activity was measured, according to the methods of Burchell et al. (5), in freeze-thawed cell suspensions containing ~30 mg cells/ml at G-6-P concentrations of (in mM) 0.5, 1, 2.5, 5, and 10. Nonspecific phosphatase activity was estimated by using paranitrophenylphosphate as substrate. Alanine aminotransferase (Sigma kit) and glutamate dehydrogenase (17) were measured spectrophoto metrically in freeze-thawed cell suspensions pretreated with 0.1% Triton X-100. GK activity was determined on aliquots of cell suspensions (~60 mg cells/ml) that were frozen in a medium containing 150 mM KCl, 50 mM HEPES, and 1.5 mg/ml dithiothreitol at pH 7.5. The aliquots were thawed and centrifuged at 20,300 g for 30 min. The supernatant was then assayed for GK activity as described by Davidson and Arion (8). All enzyme activities were determined on aliquots of cell suspensions taken before the start of incubations.

**Metabolite assays.** Glucose and lactate release into the incubation medium were measured enzymatically (Sigma kits). Glycogen was measured by using the method of Chan and Exton (6). Protein was determined by the method of Lowry et al. (16).

**Statistical procedures.** Data from each experiment were represented as the average value of each triplicate incubation. Data were analyzed using either a one-way or a two-way ANOVA where appropriate. If the overall F value obtained from the ANOVA was significant, comparisons between mean values were made by 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 groups (SU rats = 356 ± 5.9 g, ST rats = 363 ± 8.1 g). As has been previously demonstrated by using the digitonin perfusion method (7, 12), alanine aminotransferase activity was greater in PP hepatocytes, whereas glutamate dehydrogenase activi-
ity was greater in PV hepatocytes (Table 2). There were no diet effects on either alanine aminotransferase or glutamate dehydrogenase in PP and PV cell populations. Total cell yield per liver was greater from PP compared with PV hepatocyte isolations (Table 2).

Glucose release in isolated hepatocytes. In both PP and PV hepatocytes, glucagon action to increase glucose release was not different between diet groups. In PP hepatocytes, glucose release was significantly elevated in SU compared with ST at all glucagon concentrations (Fig. 1A). In PV cells, glucose release was significantly elevated in SU vs. ST at 0 and $10^{-11}$ M glucagon only (Fig. 1B). Maximal glucose release occurred at $10^{-8}$ M glucagon in all groups and was lower in ST PP cells compared with all other groups.

Glycogen degradation. Initial glycogen levels were higher in PP compared with PP cells (Fig. 2). There was no effect of diet on initial glycogen concentration or glycogenolysis (sum of glucose release and lactate accumulation) in either PP or PV hepatocytes (Fig. 3). Estimated net glycogenolysis, based on pre- to postincubation glycogen concentration, produced similar results.

Lactate accumulation. In PP cells, lactate accumulation decreased as a function of increasing glucagon concentration such that net removal of lactate was observed at $\geq 10^{-9}$ M glucagon (Fig. 4A). PV cells displayed a different pattern of lactate accumulation at low-glucagon concentrations but still exhibited net removal of lactate at $10^{-9}$ M glucagon (Fig. 4B). Under all conditions, the lactate concentration in the incubation medium was $<8 \mu M$ and endogenous in origin.

Enzyme activities. GK activity was significantly increased in ST compared with SU in both PP and PV cells (Fig. 5). In contrast to GK activity, G6Pase activity was increased in SU vs. ST in both PP and PV cells (Fig. 6). There were no differences, either between cell populations or diets, in nonspecific phosphatase activity determined by using paranitrophenylphosphate as a substrate (data not shown).

<table>
<thead>
<tr>
<th>Marker enzymes and cell yields obtained during PP and PV cell isolations from ST and SU rats</th>
<th>ST</th>
<th>SU</th>
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</thead>
<tbody>
<tr>
<td><strong>PP</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alanine aminotransferase, nmol·mg protein⁻¹·min⁻¹</td>
<td>114.5 ± 5.3*</td>
<td>66.5 ± 4.1</td>
</tr>
<tr>
<td>Glutamate dehydrogenase, nmol·mg protein⁻¹·min⁻¹</td>
<td>908.8 ± 40.8*</td>
<td>1,380.7 ± 68</td>
</tr>
<tr>
<td>Cell yield, g cells/liver</td>
<td>2.08 ±0.18*</td>
<td>1.19 ± 0.18</td>
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<tr>
<td><strong>PV</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alanine aminotransferase, nmol·mg protein⁻¹·min⁻¹</td>
<td>108.4 ±5.8*</td>
<td>70.3 ± 4.5</td>
</tr>
<tr>
<td>Glutamate dehydrogenase, nmol·mg protein⁻¹·min⁻¹</td>
<td>956.5 ± 63.3*</td>
<td>1,608.3 ± 76.3</td>
</tr>
<tr>
<td>Cell yield, g cells/liver</td>
<td>2.14 ±0.20*</td>
<td>1.26 ± 0.1</td>
</tr>
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</table>

Values are means ± SE (n = 4 cell preparations for each group). PP, periportal; PV, perivenous. *PP different from PV for a single diet group (P < 0.05).
DISCUSSION

The goal of the present study was to examine the effect of SU on glucagon-stimulated glucose release and glycogenolysis in isolated PP and PV hepatocytes. Therefore, PP and PV hepatocytes were isolated after a normal night’s feeding period. Initial glycogen concentrations in the hepatocytes obtained in the present study were 10- to 20-fold higher compared with hepatocytes obtained after a 24-h period of starvation using similar isolation procedures (4). Hepatocytes were isolated by 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 (7, 12). This pattern was characterized by increased alanine aminotransferase activity in cells isolated from the PP region and increased glutamate dehydrogenase activity in cells isolated from the PV region. Thus cell preparations designated as PP were enriched with PP cells and those designated PV were enriched with PV cells.

Data from the present study indicate that, in the absence of glucagon, glucose release was significantly increased in both PP and PV cells by SU. The increased glucose release was maintained across all glucagon concentrations in the PP cell, but only at lower glucagon concentrations in the PV cells. The only other study to examine glucose release in response to glucagon in PP and PV hepatocytes isolated from fed animals demonstrated greater glucose release in PV compared with PP cells (10). This result is consistent with the data from the ST group obtained in the present study, where the PP-to-PV ratio for glucose release was ~0.7. In contrast, this ratio was ~0.96 in the SU group. Thus SU produced a liver that was characterized by a more homogenous pattern of glucose release across the acinus but did not significantly alter the dose-response relationship between glucagon and glucose release. The increased homogeneity of glucose release in the SU group appears to be the result of adaptations in the PP population.

Increased glucose release in the absence of glucagon could result from increased glycogenolysis, increased partitioning of G-6-P toward glucose, or increased glu-

Fig. 4. Dose-dependence curves for the rate of net lactate accumulation in isolated PP (A) and PV (B) hepatocytes in response to glucagon. Data are presented as nmol lactate·h⁻¹·mg cell wet weight⁻¹. Values are means ± SE (n = 4 animals per group).

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coneogenesis. Glycogenolysis was not significantly increased in either PP or PV cells after SU feeding. In addition, the rate of glycogenolysis exceeded glucose release both in the absence of and in the presence of glucagon. Although gluconeogenesis was not estimated in the present study, the contribution of gluconeogenesis to net glucose release was probably minimal. The minimal contribution of gluconeogenesis in this study was likely because of the absence of added gluconeogenic precursors in the incubation medium. Therefore, SU appears to increase the partitioning of glycogenolysis-derived G-6-P toward glucose release.

Net glucose release from the liver is a function of the relative activities of the enzymes responsible for glucose phosphorylation (GK) and G-6-P dephosphorylation (G6Pase). In the current study, SU increased G6Pase activity in both PP and PV cell populations compared with ST. Additionally, in both the SU and ST animals, G6Pase activity was greater in the PP than in the PV population. Greater G6Pase activity in the PP compared with the PV population has been observed previously in hepatocytes isolated from 24-h-fasted rats fed SU (4). Thus a primary adaptation induced by SU is to increase G6Pase activity in the liver. It should be noted that changes in G6Pase activity were not because of effects on nonspecific phosphatases because diet effects were not observed when paranitrophenylphosphate was used as a substrate. In addition to the increased activity of G6Pase in the livers of SU animals, GK activity in both the PP and PV cell populations was lower compared with the ST group. Lower GK activity would act to augment the effect of the increased G6Pase on glucose release from the cell.

In summary, 1 wk of SU does not alter glucagon action on glycogenolysis or stimulation of glucose release in either PP or PV hepatocytes. Increased G6Pase and lower GK activity were found in both PP and PV cell populations from SU animals. Thus the greater G-6-P-to-GK activity ratio observed in both PP and PV cells from the SU animals could in part explain the greater rates of glucose release observed in the SU animals in the absence and presence of glucagon.

![Fig. 5. Glucokinase kinetics in PP (A) and PV (B) hepatocytes from ST and SU animals. Freeze-thawed cell suspensions (30 mg/ml) were assayed for glucokinase activity as described in METHODS. Data are presented as nmol·min$^{-1}$·mg protein$^{-1}$. Values are means ± SE ($n = 4$ animals per cell type per diet group). *SU significantly different from ST for a given cell population ($P < 0.05$).](http://jap.physiology.org/)

![Fig. 6. Glucose-6-phosphatase (G6Pase) kinetics in PP (A) and PV (B) hepatocytes from ST and SU animals. Freeze-thawed cell suspensions (30 mg/ml) were assayed for G6Pase activity as described in METHODS. Data are presented as nmol·min$^{-1}$·mg protein$^{-1}$. Values are means ± SE ($n = 4$ animals per cell type per diet group). *SU significantly different from ST for a given cell population ($P < 0.05$).](http://jap.physiology.org/)
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