Diets enriched in sucrose or fat increase gluconeogenesis and G-6-Pase but not basal glucose production in rats

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IN VIVO ESTIMATES SUGGEST that some prediabetic states (31, 44) and type 2 diabetes (56, 64) are characterized by increased gluconeogenesis. Accelerated gluconeogenesis in both prediabetic states and type 2 diabetes likely results from many factors, including increased precursor delivery, alterations in the hormonal milieu, and intrahepatic adaptations that increase the conversion of precursors into glucose (56, 64). In prediabetic states, increased gluconeogenesis does not result in the overproduction of glucose. This autoregulation of glucose production appears to involve reciprocal regulation of glycogenolysis (64). The development of fasting hyperglycemia and progression into type 2 diabetes likely involves an impairment in autoregulation of glucose production. Although pancreatic insufficiency would certainly contribute to the reduced ability to restrain basal glucose production in type 2 diabetes, intrahepatic adaptations such as reduced tyrosine kinase activity, increased phosphatidylinositol-4,5-bisphosphate-specific phospholipase C activity, and an increased ratio of glucose-6-phosphatase (G-6-Pase) to glucokinase have also been observed (7, 13). It has been postulated that the intrahepatic disposal of glucose 6-phosphate plays a major role in the control of glucose production and, therefore, the maintenance of autoregulation (64). Thus intrahepatic adaptations may contribute to accelerated rates of gluconeogenesis in prediabetic states and type 2 diabetes, maintenance of hepatic autoregulation in prediabetic states, and loss of autoregulation in type 2 diabetes. Presently, the sites within the liver contributing to increased gluconeogenesis and the intrahepatic adaptations that restrain glucose production are poorly understood.

Nutrients have a profound impact on hepatic glucose metabolism. In humans, intralipid infusions that acutely elevated nonesterified fatty acid (NEFA) concentrations increased gluconeogenesis and inhibited glycogenolysis (15, 61), whereas fructose administration increased gluconeogenesis without an increase in total glucose production (50, 64). The liver is also an important site of postprandial nutrient disposal. Numerous studies have demonstrated that the liver can

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account for the removal of 25–30% of an oral glucose load (19, 27, 39, 46). In addition, the liver avidly consumes dietary lipid and protein (46), and the presence of either or both of these nutrients influences the contribution of the liver to glucose removal (46). Carbohydrate composition also influences hepatic carbohydrate metabolism. For example, fructose extraction by the liver appears to be remarkably high (estimated at 50–75%), and both glucose phosphorylation and glycogen synthesis are stimulated by fructose (16, 51, 68). Thus the liver can be considered a dietary nutrient buffer with the extent of nutrient uptake by the liver determined, in large part, by the composition of nutrients presented to it. Because the development of obesity and type 2 diabetes results from both genetic and environmental factors, it would appear likely that diet composition might significantly influence hepatic glucose metabolism and thus the hepatic adaptations that characterize these disease states.

We have examined the impact of chronic changes in diet composition on hepatic glucose metabolism in rats to determine the ability of dietary nutrients to induce adaptations that characterize prediabetic states and type 2 diabetes. In rats, diets enriched in fat or 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 from a number of precursors in both perfused livers and isolated hepatocytes (10, 28, 45, 48, 49, 62). These diet-induced adaptations occur rapidly and independently of changes in body composition, are accompanied by increased hepatic lipids, and do not result in the overproduction of glucose in vivo (28, 49). Recent studies performed in humans also suggest that diets enriched in fat or protein can reduce insulin suppression of glucose production and/or increase gluconeogenesis (9, 33). One aim of the present study was to determine whether diets enriched in fat or sucrose increase gluconeogenesis in perfused liver, and reduce the ability of insulin to suppress gluconeogenesis in rats.

METABOLISM

Experimental Animals

Male Sprague-Dawley rats bred at the Arizona State University Animal Care Facility were used for this study. Rats weighed 170–210 g upon entering the study. Rats were individually caged under controlled conditions (12:12-h light-dark cycle; 50–60% relative humidity; 25°C) with free access to water. All procedures for animal use were approved by the Institutional Animal Care and Use Committee at Arizona State University.

Diet Protocol

Rats were provided ad libitum access to a semipurified starch diet (STD; Research Diets, New Brunswick, NJ) for 2 wk (baseline period) (Table 1). The baseline period was followed by an experimental diet period when rats either remained on the STD or were switched to either a high-fat diet (HFD; Table 1) or a high-sucrose diet (HSD; Table 1). To ensure equivalent energy intake, body weight gain, and body composition, rats were provided 95% of the calories consumed during the experiment period (49). Rats were studied after 7–13 days on the experimental diet. We have previously demonstrated that this duration of time results in reduced insulin suppression of glucose production in vivo (49), reduced insulin suppression of hepatic gluconeogenesis in vitro (48), and increased capacity for gluconeogenesis in vitro (10, 48) in animals fed either the HSD or HFD. Throughout the protocol, food intake was measured daily, and body weight was recorded once per week.

Experiments

In vivo gluconeogenesis. After 7–9 days of experimental diet, catheters (PE-50, Clay-Adams Intramedic) were implanted in anesthetized rats (im injection of 5 mg/kg acepromazine, 10 mg/kg xylazine, and 50 mg/kg ketamine) in the left carotid artery and right jugular vein (54). Animals were allowed 2–4 days to recover and were required to be at
NADPH-cytochrome microsomal preparation was assessed using the ratio of with the catalytic subunit (5). In addition, enrichment of the this substrate is neither bound nor transported by the glu-}

protein was determined by the Bradford method. Integrity of 1, 2, 10, and 15 mM as previously described (12). Total sodium cholate. G-6-Pase activity was measured at 0.25, 0.5, 1, 2, 10, and 15 mM as previously described (12). Total protein concentration was established, the abdomen was incised, and a portion of anesthetized. Once the absence of pedal and corneal re

Diets were formulated by Research Diets (New Brunswick, NJ). STD, high-starch diet; HSD, high-sucrose diet; HFD, high-fat diet. Maltodextrin 10 is enzyme-converted cornstarch with a dextrose equivalence of 10%. Salt and vitamin mix were prepared according to guidelines from the American Institute of Nutrition (57).

>95% of their presurgical body weight in order to be studied. Animals (n = 9–10/group) were provided their respective diets during the recovery period. On the day of study, a baseline blood sample was taken from 6- to 8-h-fasted rats, and an intravenous bolus of 14C2H2O was delivered (0.5 mCi/100 g body wt). Blood samples were taken at 30-min intervals for 4 h.

G-6-Pase activity. After 7–9 days of experimental diet, a separate group of 6- to 8-h-fasted rats (n = 6–7/group) was anesthetized. Once the absence of pedal and corneal reflexes was established, the abdomen was incised, and a portion of the liver was removed and immediately homogenized on ice in 10 mM HEPES plus 0.25 M sucrose, pH 7.4. Microsomes were extracted by the procedures of Daniele et al. (17). Briefly, the homogenate was centrifuged at 30,000 g for 10 min at 4°C. The resulting supernatant was then centrifuged for 30 min at 100,000 g at 4°C. The pellet microsomes were resuspended in 10 mM HEPES plus 0.25 M sucrose (pH 7.4). G-6-Pase activity was assessed on intact microsomes and on microsomes wherein the membrane was disrupted with 0.5% sodium cholate. G-6-Pase activity was measured at 0.25, 0.5, 1, 2, 10, and 15 mM as previously described (12). Total protein was determined by the Bradford method. Integrity of the microsomal membrane was assessed using mannose 6-phosphate (1 mM) as substrate in the G-6-Pase assay, since this substrate is neither bound nor transported by the glucose 6-phosphate transporter but has equivalent reactivity with the catalytic subunit (5). In addition, enrichment of the microsomal preparation was assessed using the ratio of NADPH-cytochrome c reductase activity in intact micro-}

subunit, p46 glucose-6-phosphate translocase) were resolved by SDS-PAGE. After electrotransfer of proteins from gels to Hybond-P membranes (Amersham Pharmacia Biotech, Piscataway, NJ), membranes were incubated with antibodies against the p36 catalytic subunit, 

Table 1. Composition of experimental diets

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<td>246</td>
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<td>200</td>
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<tr>
<td>DL-Methionine</td>
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<td>3.7</td>
</tr>
<tr>
<td>Corn starch</td>
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<td>0</td>
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<tr>
<td>Maltodextrin 10</td>
<td>184.6</td>
<td>0</td>
<td>150</td>
</tr>
<tr>
<td>Sucrose</td>
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<td>0</td>
</tr>
<tr>
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<td>50</td>
<td>50</td>
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<tr>
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</tr>
<tr>
<td>Salt mix</td>
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<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Vitamin mix</td>
<td>10</td>
<td>10</td>
<td>10</td>
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<tr>
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<table>
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<th>% energy</th>
<th>Protein</th>
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<th>Carbohydrate</th>
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<td></td>
<td>35</td>
<td>68</td>
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</table>

Diets were formulated by Research Diets (New Brunswick, NJ). STD, high-starch diet; HSD, high-sucrose diet; HFD, high-fat diet. Maltodextrin 10 is enzyme-converted cornstarch with a dextrose equivalence of 10%. Salt and vitamin mix were prepared according to guidelines from the American Institute of Nutrition (57).

Animals (n = 9–10/group) was anesthetized, and the abdominal cavity was opened to expose the portal vein, into which a catheter (20 G Angiocath, Becton-Dickson) was introduced and sutured in place. The liver was perfused using standard methods previously described (8). Trypan blue exclusion averaged 90.1 ± 1.0% and exceeded 90% for all animals except one (87%). Results were the same regardless of whether that animal was included in the analysis; thus reported data include that animal.

Initial time course studies for glucose phosphorylation and cycling. Isolated cells were suspended (30 mg/ml) in Krebs-Henseleit buffer containing 1% gelatin and incubated at 37°C (2 ml/vial, in triplicate) for 10, 20, 30, 40, 50, or 60 min in the presence of 15 mM glucose and 0.2 mM fructose with 1.5 μCi of either [2-3H]glucose (n = 3) or [6-3H]glucose (n = 3). Glucose specific activity and accumulation of H2O were determined for each rat at each time point by means of ion exchange chromatography. These studies demonstrated that accumulation of H2O was linear and that glucose specific activity remained constant over the 60-min time period for incubations with [2-3H]glucose (Fig. 1) and [6-3H]glucose (data not shown). Fructose levels were not significantly different from zero by 30–40 min of incubations. Therefore, higher fructose levels were used in subsequent studies.

Glucose phosphorylation and cycling. Isolated cells were suspended (30 mg/ml) in Krebs-Henseleit buffer containing 1% gelatin and incubated at 37°C (2 ml/vial, in triplicate) for 1 h at either 20 mM glucose or 20 mM glucose and 0.5 mM fructose. Incubations included 1.5 μCi of either [2-3H]glucose (n = 5) or [6-3H]glucose (n = 5). After 1 h, 1.0 ml of cells was added to an equal volume of perchloric acid, a portion of which was neutralized with potassium carbonate for glucose and fructose analyses and the remainder adjusted to a pH of 3 for ion exchange chromatography (9). Another portion (0.5 ml) of cells was centrifuged, the resulting supernatant was removed, and the packed cells were placed immediately into liquid N2. All samples were stored at −80°C for subsequent analysis.
Glucokinase activity and translocation. Two milliliters of suspended hepatocytes (60 mg) were incubated at 37°C for 30 min under the following conditions: 5 mM glucose, 5 mM glucose with 5 nM insulin, 20 mM glucose, 20 mM glucose with 5 nM insulin, 0.5 mM fructose, 0.5 mM fructose with 5 mM glucose, and 0.5 mM fructose with 20 mM glucose. At 30 min, hepatocytes were permeabilized in 300 mM sucrose, 3 mM HEPES, 2 mM dithiothreitol, 5 mM MgCl₂, and 0.05 mg/ml digitonin (pH 7.2), as previously described (1). After 6 min in permeabilization medium, the digitonin-treated eluate was transferred to tubes with dithiothreitol (4 mM) and immediately assayed for glucokinase activity at 50 mM glucose (18). Enzyme activity in digitonin-permeabilized cells provides an estimate of glucokinase translocated from the nucleus into the cytosol (1). The residual cell matrix was extracted in 100 mM KCl, 25 mM HEPES, 7.5 mM MgCl₂, and 0.05% Triton X-100 (−20°C for 20 min) and assayed for glucokinase activity at 50 mM glucose. A second cell matrix extraction did not result in any additional glucokinase activity.

Analytical Methods

Plasma radioactivity. Plasma samples for analysis of [3H]glucose concentration from clamp experiments were deproteinized overnight, centrifuged, and dried to eliminate [3H₂O]. Radioactivity in reconstituted samples was measured by liquid scintillation counting (Beckman Instruments, Fullerton, CA). Plasma samples for tritium incorporation into glucose from [3H₂O] were analyzed as described previously (53, 55, 58). Briefly, tritium associated with C6 of glucose was determined by periodate oxidation followed by the formation of a dimedone complex with the formaldehyde liberated from C6 (55). Tritium associated with C5 of glucose was determined after conversion of glucose to xylose and oxidation of the xylose with periodic acid (29). Tritium associated with C2 of glucose was determined after conversion of glucose 6-phosphate by use of phosphoglucose isomerase (58).

Hormones and metabolites. Plasma glucose was determined using a Beckman glucose analyzer (Fullerton, CA). Plasma NEFA were measured with the Wako NEFA C test kit (Wako Chemicals, Dallas, TX). Plasma insulin, glucagon, and corticosterone were measured by radioimmunoassay (Linco Research, St. Charles, MO). Plasma lactate and glycerol were measured fluorometrically (34).

In suspended hepatocytes, fructose concentration was determined fluorometrically (Sigma Chemical, St. Louis, MO) after removal of any glucose present with glucose oxidase and catalase. Glucose concentration was measured enzymatically using a kit (Sigma). Glycogen content and radioactivity were determined on frozen packed cells according to the method of Chan and Exton (14). Protein determinations were made using the Bradford method.

Ion exchange chromatography on samples from suspended hepatocytes. Samples (0.5 ml) were placed onto a series of two columns (hydrogen resin, Bio-Rad AG 50W-X8 and acetate resin, Bio-Rad AG1-X8). Glucose was eluted from the initial water rinse. Amino acids were eluted off the hydrogencolumn with 4 M NH₄. Lactate and pyruvate were eluted off the acetate column with 2 M formic acid and 2 M acetic acid, respectively. [3H₂O] accumulation was determined from aliquots of the glucose fraction that were counted directly or evaporated to dryness. Lactate, pyruvate, and amino acid standards were used to assess recovery.

Calculations

In vivo studies. Tracer-estimated glucose appearance was calculated by isotope dilution with adjustment for the labeled endogenous infusate (20, 60). Gluconeogenesis was estimated from the ratio of tritium bound to C6 of glucose relative to that bound to C2 of glucose (58). This method takes advantage of the binding of [3H] from [3H₂O] to C3 of pyruvate that becomes C6 of glucose in the gluconeogenic process (29, 58). Because the [3H] bound to C2 of glucose arises from both gluconeogenesis and glycogenolysis, the amount of [3H] bound to C6 relative to that bound to C2 provides an estimate of glucose formed via nonglycerol gluconeogenesis (29). Total gluconeogenesis was calculated using the ratio of tritium bound to C5 of glucose relative to that bound to C2 (30).

In vitro studies. Rates of glucose phosphorylation were calculated from the sum of accumulated [3H₂O] and tritiated glycogen from vials containing [2-3H]glucose (24). Estimation of tritiated glycogen served to decrease the error resulting from incomplete equilibration between glucose 6-phosphate and fructose 6-phosphate (26). Glycolysis was calculated as the sum of tritiated pyruvate, lactate, amino acids, and tritiated water in vials containing [6-3H]glucose (24). Glucose cycling was calculated as the difference between glucose phosphorylation and total glucose metabolism (glycolysis + [6-3H]glucose incorporation into glycogen) (24). Thus glucose cycling represents phosphorylated glucose not further metabolized either glycolytically or into glycogen (24). Net gluconeogenesis was estimated from the incorporation of [6-3H]glucose into glycogen. Assumptions regarding these techniques have been discussed in detail previously (24, 26).

Enzymes. Total glucokinase activity from isolated cells was calculated as the sum of glucokinase activity in the digitonin eluate and that in the residual cell matrix (1). Microsomal membrane integrity is presented as “percent latency of mannose-6-phosphatase,” calculated as: 100 − [100 × (mannose-6-phosphatase activity in intact microsomes)/(mannose-6-phosphatase activity in disrupted microsomes)] (5). Thus 100% latency would represent zero activity in intact microsomes and, therefore, an inability of mannose-6-phosphate to gain access to the catalytic subunit.

Data Analysis

Data were analyzed both as a one-way analysis of variance (ANOVA) using Bonferroni’s multiple comparison test to determine where significant differences lie and by the non-parametric analysis of Kruskal-Wallis. The two analyses gave identical interpretations. Student’s unpaired t-test was used to compare within-diet responses to different experimental conditions. Significance was set at P ≤ 0.05. All data are reported as means ± SE.

Fig. 1. [3H₂O] accumulation (dpm/ml) and glucose specific activity (dpm/μmol) in hepatocytes provided with 15 mM glucose and 0.2 mM fructose. Representative experiment using [2-3H]glucose.
RESULTS

Energy Intake and Weight Gain

During the experimental diet feeding period, energy intake (−100 kcal/day) and body weight gain (−40 g/wk) were not significantly different among diet groups.

In Vivo Gluconeogenesis

Because we had previously demonstrated that both the HSD and HFD increased the capacity for gluconeogenesis in perfused liver and isolated hepatocytes from a number of precursors (10, 48), this first experiment sought to determine whether this adaptation occurred in vivo.

There were no significant differences among diet groups in any of the metabolites or hormones measured (Table 2). Nonglycerol gluconeogenesis, as assessed by the C6:C2 ratio of glucose, was significantly increased in HSD and HFD (Table 2). In addition, total gluconeogenesis, as assessed by the C5:C2 ratio, was also increased in HSD and HFD (Table 2). The tritiated water pool appeared to reach steady state between 30 and 60 min after injection (data not shown).

G-6-Pase Activity and Protein Levels

In a previous study, short-term (1 wk) exposure to the HSD increased G-6-Pase activity in whole cell homogenates isolated from periporal or perivenous regions of the liver (11). In this experiment, we sought to determine whether diet-induced changes in G-6-Pase activity involved the microsomal membrane and the extent to which HSD and HFD influenced the catalytic subunit and glucose-6-phosphate translocase protein.

G-6-Pase activity was significantly increased in both intact and disrupted microsomes from HSD and HFD rats (Fig. 2). Consistent with the observed diet-induced increase in G-6-Pase activity, Western blot analysis demonstrated that both the HSD and HFD increased (P < 0.05) the amount of the p36 catalytic subunit (Fig. 3). In contrast, only the HSD produced a significant increase in the amount of the p46 glucose-6-phosphate translocase (Fig. 3). Insulin and glucose concentrations were not significantly different among diet groups (Table 3). Neither percent latency nor the enrichment of NADPH-cytochrome c reductase (microsomal marker) was significantly different among groups (Table 3).

Table 2. Plasma metabolites, hormones, and gluconeogenesis in fasted rats

<table>
<thead>
<tr>
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<th>STD (n = 9)</th>
<th>HSD (n = 9)</th>
<th>HFD (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin, µU/ml</td>
<td>30 ± 4</td>
<td>35 ± 5</td>
<td>34 ± 5</td>
</tr>
<tr>
<td>Glucagon, pg/ml</td>
<td>97 ± 11</td>
<td>103 ± 12</td>
<td>91 ± 10</td>
</tr>
<tr>
<td>Corticosterone, ng/ml</td>
<td>132 ± 21</td>
<td>152 ± 24</td>
<td>115 ± 19</td>
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<tr>
<td>Glucose, mM</td>
<td>6.1 ± 0.4</td>
<td>6.3 ± 0.5</td>
<td>6.2 ± 0.4</td>
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<tr>
<td>Lactate, mM</td>
<td>0.9 ± 0.1</td>
<td>0.7 ± 0.2</td>
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<tr>
<td>Glycerol, mM</td>
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<td>0.18 ± 0.01</td>
<td>0.16 ± 0.02</td>
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<tr>
<td>NEFA, mM</td>
<td>0.7 ± 0.1</td>
<td>0.8 ± 0.2</td>
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</tr>
<tr>
<td>C6:C2</td>
<td>0.40 ± 0.10</td>
<td>0.68 ± 0.07*</td>
<td>0.71 ± 0.08*</td>
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<tr>
<td>C5:C2</td>
<td>0.49 ± 0.06</td>
<td>0.79 ± 0.08*</td>
<td>0.81 ± 0.07*</td>
</tr>
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</table>

Values are means ± SE. NEFA, nonesterified fatty acids. C6:C2, tritium incorporation from 3H2O into the 6th carbon of glucose relative to that in the 2nd carbon of glucose; C5:C2, tritium incorporation from 3H2O into the 5th carbon of glucose relative to that in the 2nd carbon of glucose. Higher ratio indicates a greater contribution of nonglycerol gluconeogenesis to glucose production or total gluconeogenesis to glucose production, respectively. *Significantly different from STD.

In Vivo Glucose Appearance Under Basal and Hyperinsulinemic Conditions

We investigated basal and insulin-stimulated glucose metabolism in the three dietary groups to demonstrate that these diets, when provided for 1 wk, do not increase basal glucose production but do lead to insulin resistance on glucose production.

Insulin, glucose, and NEFA concentrations were not significantly different among diet groups (Table 4). Basal rates of glucose appearance were not significantly different among diet groups (Table 4). Insulin suppression of NEFA concentrations was not significantly different among groups (Table 4). However, insulin suppression of endogenous glucose appearance was significantly reduced in HSD and HFD (Table 4).
tent (mg/g wet cells) was low and not significantly different among diets and never reached zero (i.e., below the level of detection by assay) in any vial. Glycogen concentration (mM) did not change significantly over the 1-h incubation period to 0.11 in HFD, which fructose was present, concentrations (mM) fell rapidly with 20 mM glucose (Table 5). Although both glucose phosphorylation and cycling tended to be higher in HSD after incubations with glucose and fructose, differences did not reach statistical significance (Table 5). Glycolysis and tracer incorporation into glycogen were not significantly different among diet groups (data not shown).

Low concentrations of fructose are known to stimulate glucose phosphorylation (41). Because we did not observe fructose stimulation of glucose phosphorylation at 20 mM glucose, we estimated glucose phosphorylation in a subset of rats (n = 3–4/diet) at lower glucose concentrations in the presence and absence of fructose. Under these conditions, glucose phosphorylation was increased by fructose (HSD, 38%; HFD, 42%; STD, 46%; Fig. 4).

Glucokinase Translocation

Glucokinase activity in digitonin eluates was significantly higher in HSD-fed rats after incubations with either 5 or 20 mM glucose (Fig. 5). This was the case whether the data were reported as an absolute rate (Fig. 5) or as a percentage of the total activity (data not shown). The glucose-induced increment (from 5 to 20 mM glucose) in glucokinase activity in digitonin eluates was not significantly different among diets (Fig. 5). Glucokinase activity in digitonin eluates was not significantly different among diet groups with fructose alone, and the increment in glucokinase activity resulting from fructose addition to either low or high glucose was significantly less in HSD-fed rats vs. HFD- and STD-fed rats (Table 6). Glucokinase activity in digitonin eluates after incubations with insulin was significantly reduced in HSD at both 5 and 20 mM glucose and in HFD at 20 mM glucose vs. STD (Fig. 6).

Within each diet group, total glucokinase activity (sum of digitonin eluate and residual cell matrix) was not significantly different across substrate conditions and averaged 12% higher in HSD compared with STD and HFD. Western blot analysis of total cell lysates from livers of 6- to 8-h-fasted rats demonstrated that glucokinase protein levels were not significantly different among diet groups (data not shown).

DISCUSSION

The present study examined the effects of short-term (≈1 wk) exposure to diets enriched with sucrose or fat on in vivo gluconeogenesis, the catalytic and glucose-6-phosphate translocase subunits of G-6-Pase, and glucokinase. The results demonstrate that short-term exposure to either the high-sucrose or the high-fat diet increased the contribution of gluconeogenesis to glucose production in vivo without apparent effects on the circulating environment. These diets also increased microsomal G-6-Pase activity via changes in the fraction of the catalytic and glucose-phosphorylation and cycling. Glucose phosphorylation and glucose cycling were significantly higher in HSD compared with STD after incubations with 20 mM glucose (Table 5). Although both glucose phosphorylation and cycling tended to be higher in HSD after incubations with glucose and fructose, differences did not reach statistical significance (Table 5). Glycolysis and tracer incorporation into glycogen were not significantly different among diet groups (data not shown).

**Table 3. Integrity of microsomal preparations**

<table>
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<tr>
<th></th>
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<th>HSD</th>
<th>HFD</th>
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<tr>
<td>Plasma glucose, mM</td>
<td>6.1 ± 0.3</td>
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<td>Plasma insulin, µU/ml</td>
<td>33 ± 6</td>
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<tr>
<td>reductase ratio</td>
<td>5.6 ± 0.2</td>
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<td>Percent latency</td>
<td>92.8 ± 2.1</td>
<td>93.8 ± 1.6</td>
<td>93.9 ± 1.6</td>
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Values are means ± SE; for n = 6–7/group. Glucose and insulin samples from cardiac puncture. See METHODS for definitions of cytochrome c reductase ratio and percent latency.
The amount of the p36 catalytic subunit. Exposure to the sucrose diet also increased the amount of the p46 glucose-6-phosphate translocase protein, although this did not result in any further increase in G-6-Pase activity in intact microsomes. Although both the high-sucrose and high-fat diets increased gluconeogenesis and G-6-Pase, normal rates of glucose production and glycemia were maintained that were not dependent on compensatory hyperinsulinemia (i.e., basal insulin levels were not significantly different among diet groups). Thus intrahepatic adaptations that might serve to offset the diet-induced increase in G-6-Pase were explored. The results demonstrate that exposure to the high-sucrose diet increased glucose phosphorylation, glucose cycling, and the activity of glucokinase in digitonin-treated eluates from hepatocyte cell suspensions incubated with basal and elevated glucose concentrations.

We have previously demonstrated that exposure to sucrose- or fat-enriched diets for 1 wk increased the capacity for gluconeogenesis in both isolated hepatocytes (10) and perfused livers (48). One of the goals of the present study was to determine whether this adaptation was retained in vivo. Toward this end, we assessed the incorporation of tritiated water into glucose to estimate both nonglycerol gluconeogenesis (C6:C2 ratio) and total gluconeogenesis (C5:C2 ratio) in 6- to 8-h-fasted, conscious rats. Both the C5:C2 and the C6:C2 ratios were significantly increased in high-sucrose and high-fat diets compared with the high-starch diet. Thus diets enriched in sucrose or fat increase both the capacity for gluconeogenesis in vitro and the contribution of gluconeogenesis to glucose production in vivo. Importantly, in vivo changes in gluconeogenesis occurred under conditions in which circulating substrate and hormone concentrations were not significantly different among groups. Thus intrahepatic adaptations to high-sucrose and high-fat diets can lead to increased reliance on gluconeogenesis in vivo.

The intrahepatic sites that appear to contribute to these diet-induced increases in gluconeogenesis include pyruvate carboxylase, phosphoenolpyruvate carboxykinase (PEPCK), and fructose-1,6-bisphosphatase (10, 59). Our previous data suggested that the increased capacity for flux through pyruvate carboxylase and PEPCK was, in large part, the result of increased intrahepatic lipid oxidation (10). The terminal enzyme of both gluconeogenesis and glycogenolysis in the liver, G-6-Pase, appears to respond to changes in nutrient composition/delivery (4, 11, 35–38). However, previous studies were all performed in the absence of insulin (in vitro) or in the presence of basal insulin concentrations (in vivo). The repressive effects of insulin on G-6-Pase transcription and mRNA have been shown to be dominant over the stimulatory affects of cAMP and dexamethasone.

Table 4. Basal and euglycemic hyperinsulinemic clamp results

<table>
<thead>
<tr>
<th></th>
<th>STD (n = 5)</th>
<th>HSD (n = 6)</th>
<th>HFD (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal conditions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma glucose, mM</td>
<td>6.3 ± 0.5</td>
<td>6.5 ± 0.4</td>
<td>6.5 ± 0.5</td>
</tr>
<tr>
<td>Plasma insulin, μU/ml</td>
<td>35 ± 6</td>
<td>39 ± 5</td>
<td>41 ± 6</td>
</tr>
<tr>
<td>Glucose appearance, mg·kg⁻¹·min⁻¹</td>
<td>12.8 ± 1.2</td>
<td>12.5 ± 1.0</td>
<td>12.9 ± 0.9</td>
</tr>
<tr>
<td>Hyperinsulinemic, euglycemic conditions</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma glucose, mM</td>
<td>6.5 ± 0.6</td>
<td>6.4 ± 0.4</td>
<td>6.6 ± 0.5</td>
</tr>
<tr>
<td>Plasma insulin, μU/ml</td>
<td>146 ± 11</td>
<td>155 ± 15</td>
<td>143 ± 12</td>
</tr>
<tr>
<td>Plasma NEFA, mM</td>
<td>0.51 ± 0.04</td>
<td>0.29 ± 0.04</td>
<td>0.26 ± 0.03</td>
</tr>
<tr>
<td>Glucose infusion rate, mg·kg⁻¹·min⁻¹</td>
<td>15.4 ± 1.2</td>
<td>10.4 ± 1.0*</td>
<td>9.4 ± 0.9*</td>
</tr>
<tr>
<td>Endogenous glucose appearance, mg·kg⁻¹·min⁻¹</td>
<td>4.1 ± 0.5</td>
<td>9.4 ± 0.7*</td>
<td>9.1 ± 0.8*</td>
</tr>
<tr>
<td>Total glucose disappearance, mg·kg⁻¹·min⁻¹</td>
<td>19.5 ± 1.3</td>
<td>19.8 ± 1.1</td>
<td>18.5 ± 1.1</td>
</tr>
</tbody>
</table>

Values are means ± SE. Basal conditions represent the average of 3 samples taken between 100 and 120 min. Hyperinsulinemic euglycemic conditions represent the average of 3 samples taken between 220 and 240 min. *Significantly different from STD.

Table 5. Tracer-estimated glucose metabolism in isolated hepatocytes

<table>
<thead>
<tr>
<th></th>
<th>STD</th>
<th>HSD</th>
<th>HFD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose phosphorylation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose, 20 mM</td>
<td>1.3 ± 0.3</td>
<td>2.2 ± 0.2*</td>
<td>1.8 ± 0.3</td>
</tr>
<tr>
<td>Glucose, 20 mM + fructose, 0.5 mM</td>
<td>1.8 ± 0.2</td>
<td>2.2 ± 0.4</td>
<td>1.9 ± 0.4</td>
</tr>
<tr>
<td>Glucose cycling</td>
<td>0.2 ± 0.3</td>
<td>0.9 ± 0.2*</td>
<td>0.6 ± 0.3</td>
</tr>
<tr>
<td>Glucose, 20 mM</td>
<td>0.2 ± 0.2</td>
<td>0.7 ± 0.4</td>
<td>0.4 ± 0.5</td>
</tr>
<tr>
<td>Glucose, 20 mM + fructose, 0.5 mM</td>
<td>0.2 ± 0.2</td>
<td>0.7 ± 0.4</td>
<td>0.4 ± 0.5</td>
</tr>
</tbody>
</table>

Values are means ± SE in nmol·mg cell⁻¹·min⁻¹; n = 5/group. *Significantly different from STD.

Fig. 4. Glucose phosphorylation at low glucose (GLC) concentrations with or without fructose (FRU). Values are means ± SE; n = 3–4/diet group. *Significantly greater than 5.5 mM glucose alone.
methasone (63). In the present study, the consumption of diets enriched in sucrose or fat for ~1 wk increased the amount of the p36 catalytic subunit of G-6-Pase. Because the delivery of nutrients was accomplished by ingestion, their presentation to the liver occurred in the context of hyperinsulinemia. Thus, if the diet-induced increase in G-6-Pase catalytic subunit protein requires increased gene expression, it suggests that these dietary nutrients or specific aspects of their metabolism overcome the dominant effects of insulin, that the regulation of G-6-Pase by chronic nutrient delivery is distinct from that which occurs acutely, and/or that increased protein expression results from nutrient-induced adaptations in posttranscriptional events.

Although streptozotocin-induced diabetes has been shown to increase both the catalytic subunit and the glucose-6-phosphate translocase (32), the latter appears to be less responsive to hypoinsulinemia in vivo, hyperinsulinemia in H4IIE cells, and cAMP stimulation in both H4IIE cells and primary hepatocytes (25). In the present study, exposure to the sucrose-enriched diet increased protein levels for both the catalytic subunit and the glucose-6-phosphate translocase, whereas the high-fat diet increased the amount of the catalytic subunit protein only. Importantly, upregulation of the glucose-6-phosphate translocase after sucrose exposure did not result in increased microsomal G-6-Pase activity relative to the high-fat diet group. These data suggest that compensatory upregulation of the translocase, to match changes in the catalytic subunit, is not required to achieve adequate delivery of glucose 6-phosphate to the catalytic subunit in intact microsomes.

G-6-Pase catalytic subunit mRNA, protein, and activity are subject to regulation by hormones and nutrients (4, 25, 35–38, 63, 67). In particular, incubation of liver microsomes with polyunsaturated fatty acyl-CoA esters decreased G-6-Pase activity, whereas G-6-Pase activity was increased when microsomes were incubated with saturated fatty acyl-CoA esters (41, 42). In the present study, continuous provision of a high-polyunsaturated fatty acid diet for ~1 wk increased the amount of the catalytic subunit and the activity of G-6-Pase in both intact and disrupted microsomes. Two previous studies have reported that high-polyunsaturated fatty acid diets provided for periods up to or exceeding 3 wk resulted in decreased microsomal G-6-Pase activity (22, 40). The reasons for this discrepancy are not obvious but may include the duration of dietary treatment, differences in overall nutrient composition of the diets, and the type of polyunsaturated fatty acid used. We have previously demonstrated that high-sucrose diets increase the saturated fatty acid content of plasma and microsomal membranes in the liver (52). Thus it is possible that the sucrose diet-mediated induction of the catalytic and/or translocase subunits involves changes to the membrane lipid environment.

Table 6. Glucokinase activity in digitonin eluates

<table>
<thead>
<tr>
<th>Condition</th>
<th>STD</th>
<th>HSD</th>
<th>HFD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low glucose, 5 mM</td>
<td>9 ± 2</td>
<td>32 ± 4*</td>
<td>4 ± 1</td>
</tr>
<tr>
<td>High glucose, 20 mM</td>
<td>87 ± 9</td>
<td>146 ± 6*</td>
<td>83 ± 10</td>
</tr>
<tr>
<td>Fructose, 0.5 mM</td>
<td>52 ± 4</td>
<td>59 ± 2</td>
<td>47 ± 5</td>
</tr>
<tr>
<td>Low glucose, 5 mM + fructose, 0.5 mM</td>
<td>95 ± 9</td>
<td>90 ± 10†</td>
<td>83 ± 5</td>
</tr>
<tr>
<td>High glucose, 20 mM + fructose, 0.5 mM</td>
<td>136 ± 8</td>
<td>144 ± 4‡</td>
<td>114 ± 5</td>
</tr>
</tbody>
</table>

Values are means ± SE in nmol mg−1 protein−1 min−1; n = 5–6/group. *Significantly different from STD and HFD; †increment from low glucose significantly different from STD; ‡increment from high glucose significantly different from STD.

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The overexpression of G-6-Pase produces glucose intolerance but not fasting hyperglycemia (65). The explanation for such an observation may involve the low control strength exerted by G-6-Pase on glucose production (2). In the present study, the high-sucrose and high-fat diets not only increased G-6-Pase but also gluconeogenesis, yet normal rates of basal glucose production and concentrations of glucose and insulin were maintained. The maintenance of normal rates of glucose production in these dietary models likely results from appropriate adjustments in glycogenolysis. However, it is interesting that basal phosphatidylinositol (PI) 3-kinase activity in the liver was increased after exposure to the high-sucrose diet (47), whereas high-fat diets appear to increase the amount of p85 associated with insulin receptor substrate (IRS)-1 in the basal state (3).

The net release of glucose represents the balance between G-6-Pase and glucokinase activities. Thus diet-induced adaptations in glucokinase might serve to offset upregulation of G-6-Pase. The regulation of glucokinase is complex, involving a regulatory protein and translocation from the nucleus to the cytoplasm in response to various stimuli (1, 43, 66). The digitonin release assay measures the distribution of glucokinase between free and bound states (1). The latter most likely represents glucokinase bound to GKRP (43). Recent work comparing this assay with glucokinase translocation determined by immunofluorescence demonstrated comparable results (43). In the present study, glucokinase activity in digitonin eluates was increased in hepatocytes isolated from sucrose-fed rats at both low (5 mM) and high (20 mM) glucose concentrations. In addition, glucose phosphorylation and glucose cycling were significantly increased in hepatocytes from sucrose-fed rats. These data support the notion that translocation of glucokinase out of the cell nucleus was promoted and/or import back into the nucleus was restricted in hepatocytes isolated from rats fed the high-sucrose diet. The result of this sucrose-induced adaptation is increased glucose phosphorylation and cycling. The lack of effect of the high-fat diet on glucokinase and glucose phosphorylation may relate to the potent ability of fructose to regulate this protein (66, 68). In a recent study, the infusion of small amounts of fructose corrected the regulation of glucose production by hyperglycemia in type 2 diabetic subjects, suggesting that impaired regulation of glucokinase may contribute to the dysregulation of glucose production in this disease (23).

Glucokinase activity in digitonin-treated eluates was higher in high-sucrose diet-fed rats in the absence of insulin. Addition of insulin resulted in glucokinase activities in digitonin-treated eluates that were not significantly different from the high-starch diet-fed rats. Thus it is possible that glucokinase translocation approached a maximum in the high-sucrose diet group in the absence of insulin. Alternatively, the adaptation leading to increased glucokinase translocation in high-sucrose diet-fed rats may involve steps that are also used by insulin. However, the first possibility does not explain the lack of insulin stimulation of glucokinase translocation at low glucose concentrations, where adequate room should be available to expand glucokinase activity in digitonin-treated eluates. The second possibility requires further study.

The ability of insulin to suppress glucose production in vivo and stimulate glucokinase translocation in vitro was reduced in both high-sucrose and high-fat diets. It appears that sucrose-induced hepatic insulin resistance involves impairments in insulin-stimulated tyrosine phosphorylation of IRS proteins and activation of PI 3-kinase, whereas impairments downstream of PI 3-kinase characterize high-fat diet-induced hepatic insulin resistance (3, 47). It is worth noting that both of these diets produced hepatic insulin resistance without affecting insulin-stimulated glucose disappearance or suppression of NEFA levels. In fact, the development of peripheral insulin resistance requires 2–3 wk of diet exposure and is accompanied by fasting hyperinsulinemia (28, 49).

The present study examined the effects of diets enriched with sucrose or fat on in vivo gluconeogenesis, glucose-6-phosphatase, and glucokinase translocation. The data demonstrate that relatively short-term chronic exposure to these diets increased in vivo gluconeogenesis, the expression of the catalytic subunit of glucose-6-Pase, and G-6-Pase activity in both intact and disrupted microsomes. Diets enriched with sucrose also increased the expression of the p46 glucose-6-phosphate translocase. However, this additional adaptation did not result in a further increase in microsomal G-6-Pase activity. Sucrose exposure also led to increased cytosolic availability of glucokinase and an increased capacity for glucose phosphorylation. These latter adaptations may serve to compensate for upregulation of G-6-Pase after sucrose exposure.

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REFERENCES


