Hyperglycemia compensates for diet-induced insulin resistance in liver and skeletal muscle of rats

S. RENEE COMMERFORD, MICHAEL E. BIZEAU, HEATHER McRAE, AMI JAMPOLIS, JEFFREY S. THRESHER, AND MICHAEL J. PAGLIASSOTTI

Arizona State University, Exercise Science Research Institute, Tempe, Arizona 85287-0404

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Commerford, S. Renee, Michael E. Bizeau, Heather McRae, Ami Jampolis, Jeffrey S. Thresher, and Michael J. Pagliassotti. Hyperglycemia compensates for diet-induced insulin resistance in liver and skeletal muscle of rats. Am J Physiol Regulatory Integrative Comp Physiol 281: R1380–R1389, 2001.—High-fat and high-sucrose diets increase the contribution of gluconeogenesis to glucose appearance (glc R) under basal conditions. They also reduce insulin suppression of glc R, and insulin-stimulated muscle glucose synthesis under euglycemic, hyperinsulinemic conditions. The purpose of the present study was to determine whether these impairments influence liver and muscle glycogen synthesis under hyperglycemic, hyperinsulinemic conditions. Male rats were fed a high-sucrose, high-fat, or low-fat, starch control diet for either 1 (n = 5–7/group) or 5 wk (n = 5–6/group). Studies involved two 90-min periods. During the first, a basal period (BP), [6-3H]glucose was infused. In the second, a hyperglycemic period (HP), [6-3H]glucose, [6-14C]glucose, and unlabeled glucose were infused. Plasma glucose (BP: 111.2 ± 1.5 mg/dl; HP: 172.3 ± 1.5 mg/dl), insulin (BP: 2.5 ± 0.2 ng/ml; HP: 4.9 ± 0.3 ng/ml), and glucagon (BP: 81.8 ± 1.6 ng/l; HP: 74.0 ± 1.3 ng/l) concentrations were not significantly different among diet groups or with respect to time on diet. There were no significant differences among groups in the glucose infusion rate (mg·kg⁻¹·min⁻¹) necessary to maintain arterial glucose concentrations at ~170 mg/dl (pooled average: 6.4 ± 0.8 at 1 wk; 6.4 ± 0.7 at 5 wk), percent suppression of glc R (44.4 ± 7.8% at 1 wk; 63.2 ± 4.3% at 5 wk), tracer-estimated net liver glucose synthesis (7.8 ± 1.3 µg·g liver⁻¹·min⁻¹ at 1 wk; 10.5 ± 2.2 µg·g liver⁻¹·min⁻¹ at 5 wk), indirect pathway glucose synthesis (3.7 ± 0.9 µg·g liver⁻¹·min⁻¹ at 1 wk; 3.4 ± 0.9 µg·g liver⁻¹·min⁻¹ at 5 wk), or tracer-estimated net muscle glycogen synthesis (1.0 ± 0.3 µg·g muscle⁻¹·min⁻¹ at 1 wk; 1.6 ± 0.3 µg·g muscle⁻¹·min⁻¹ at 5 wk). These data suggest that hyperglycemia compensates for diet-induced insulin resistance in both liver and skeletal muscle.

LIVER GLUCOSE METABOLISM is impaired in type 2 diabetes mellitus. This impairment involves both the fasted state, where gluconeogenesis appears to be increased (3, 15), and the fed state, where insulin suppression of glucose production is reduced (5). Both genetic and environmental factors contribute to the development of these impairments. Diet composition represents one environmental factor that can influence both gluconeogenesis and insulin action on glucose production (22–27). For example, both high-fat (HFD) (27) and high-sucrose (HSD) (unpublished observations) diets increase the contribution of gluconeogenesis to glucose production under fasted, basal conditions. In addition, these diets reduce insulin suppression of glucose production under hyperinsulinemic, euglycemic conditions (24, 25, 39). Thus changes in diet composition can produce a liver that is more gluconeogenic and resistant to insulin suppression.

In the postprandial state, the liver removes ~30% of an oral glucose load (6, 7, 13) with a significant proportion being directed to glycogen (16), both by the direct (glucose → glycogen) and indirect (glucose → 3-carbon intermediates → glucose-6-phosphate → glycogen) pathways (17, 30, 34, 35). It is reasonable to predict that a liver characterized by insulin resistance and increased reliance on gluconeogenesis would, in the postprandial state, undergo lower rates of glycolysis synthesis and/or synthesize a greater proportion of its glycogen via indirect pathway glycogen synthesis. Indeed, a high-protein diet produced elevated rates of basal glucose production largely due to an increased rate of gluconeogenesis and an increase in the contribution of the indirect pathway to glycogen synthesis (32). The present study was designed to test the hypothesis that, under conditions of liver glucose uptake (i.e., hyperglycemia and hyperinsulinemia), net glycolysis synthesis would be reduced and the contribution of indirect pathway glycogen synthesis would be greater in HSD and HFD rats compared with low-fat, high-starch diet (STD)-fed rats.

METHODS

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 on 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; Table 1) for 2 wk (baseline period). The baseline period was followed by an experimental diet period where rats either remained on the STD or were switched to either HFD (Table 1) or HSD (Table 1). To ensure equivalent energy intake, body weight gain, and body composition, rats were provided 95% of the calories consumed during the second week of ad libitum baseline feeding throughout the experimental diet period. Rats were studied after 1 or 5 wk of experimental diet feeding. We chose to study rats at different lengths of time on diet for several reasons: 1) gluconeogenesis is increased after only 1 wk of HFD or HSD feeding, 2) HSD and HFD produce hepatic insulin resistance after 1 wk of feeding (24), and 3) these diets produce skeletal muscle insulin resistance after 2–5 wk (19, 24, 39). Throughout the protocol, food intake was measured daily, and body weight was recorded once per week.

**Animal Preparation**

After either 1 or 5 wk of experimental diet feeding and several days before an animal's day of study, catheters were implanted into the jugular vein (tracer infusions) and carotid artery (blood sampling) while the rats were under general anesthesia as previously described (28). Rats were allowed to recover for at least 4 days before being studied. Rats ate their respective diets during recovery. The body weight of rats used in the studies was ≈94% of presurgery weight.

**Basal and Hyperglycemic Clamps**

At 0900 the morning before the day of study, body weight and the previous day's food intake were measured, and all food was removed. Studies commenced between 0900 and 1200 the following morning so that all animals were fasted 24–27 h at the start of the study. On the morning of the study day, extensions were placed onto exteriorized catheters to allow easy access. Before initiating infusions, an arterial blood sample was drawn to determine 24-h fasted (basal) glucose concentrations. Rats (n = 5–7/diet group per time) were studied only if basal glucose concentrations were below 130 mg/dl. To estimate basal glucose kinetics, a primed (3.0 μCi) continuous infusion (0.1 μCi/min) of [6-3H]glucose was initiated (American Radiolabeled Chemicals, St. Louis, MO) into the venous catheter. Arterial blood samples were collected at 70, 80, and 90 min of tracer infusion to determine 3H glucose-specific activity (glc SA). Plasma insulin and glucagon concentrations were determined from the 90-min blood sample only. At t = 90, rats were momentarily disconnected from the infusion pump, and a bolus of tracer containing [6-14C] and [6-3H]glucose (3.2 and 4.3 μCi, respectively) in 100 μl of saline was injected through the venous extension. Rats were then reconnected to the infusion pump, and a constant infusion of [6-14C] and [6-3H]glucose was begun (0.13 and 0.16 μCi/min, respectively). At the same time, a variable infusion of unlabeled glucose (20%) was started via the venous catheter to achieve an arterial glucose concentration of ≈170 mg/dl (hyperglycemic period). This concentration of glucose was maintained for a period of 90 min (90–180 min). Arterial samples were taken at 5- to 10-min intervals, and the infusion of unlabeled glucose was adjusted accordingly. 3H and 14C glc SAs were determined from blood samples taken at t = 105, 120, 135, 160, 170, and 180 min. Plasma insulin and glucagon were determined from blood samples taken at t = 135 and 180 min only. After the last blood sample (180 min), pentobarbital sodium (50 mg/kg iv) was administered, and a blood sample from the portal vein was taken while infusions were maintained. A small portion of the liver was then removed and immediately placed into liquid N2. The remainder of the liver and the gastrocnemius muscle were taken, weighed, and placed into liquid N2. The epididymal, retroperitoneal, and mesenteric fat pads were then weighed and discarded. Urine was removed from the bladder, total urine volume was recorded, and urinary glucose and [3H] and [14C]glucose concentrations were measured. The harvesting of blood and tissues after death took less than 2 min.

**Basal and Euglycemic Clamp Studies**

On a separate group of rats (n = 4/diet group per time), studies were performed exactly as described above with the exception of the second 90-min period (90–180 min). At t = 90, a primed continuous infusion of insulin (3.1 mU·kg−1·min−1) was begun, and the [3-3H]glucose infusion rate (GIR) was increased to 0.15 μCi/min to minimize changes in plasma glc SA from the basal to the hyperinsulinemic period. At the same time, an exogenous infusion of 10% dextrose was administered venously at variable rates to maintain euglycemia. Arterial blood samples (∼30 μl) were taken throughout the subsequent 90 min at 5- to 10-min intervals to determine the exogenous GIR necessary to maintain euglycemia. Three arterial samples (∼200 μl) were taken at 10-min intervals during the final 30-min steady-state period for determination of glucose concentration, insulin (final sample only), and glucose radioactivity. Animals were killed, and tissues and blood samples were taken as described above.

**Basal Studies**

A separate group of rats (n = 3–4/diet per time on diet) was anesthetized following the 90-min basal period. Blood and tissue sampling were done exactly as described above. Basal studies were not statistically compared because of the small group sizes. These studies were performed primarily to assure there were no marked differences in certain metabolic processes of interest (basal glc Ra, hepatic glycogenesis) among groups before the experimental manipulation of hyperglycemia.

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**Table 1. Composition of experimental diets**

<table>
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<tr>
<th></th>
<th>HFD</th>
<th>HSD</th>
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<td>Casein</td>
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<td>200</td>
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<tr>
<td>DL-methionine</td>
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<tr>
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<tr>
<td>Vitamin mix</td>
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<td>10</td>
<td>10</td>
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<tr>
<td>Choline bitartrate</td>
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Diet composition given in g/kg. Diets were formulated by Research Diets (New Brunswick, NJ). HFD, high-fat diet; HSD, high-sucrose diet; STD, low-fat, high-starch diet. Maltodextrin 10 is enzyme-converted corn starch with a dextrose equivalence of 10%. Salt and vitamin mixes are prepared according to guidelines from the American Institute of Nutrition (31).
Analytic Methods

Plasma and tissue radioactivity. Plasma tracer samples were deproteinized (37) with equal volumes of barium hydroxide and zinc sulfate (0.03 N) and stored overnight at 4°C. To determine glucose radioactivity, a portion of the supernatant was dried and reconstituted in distilled deionized water. In studies involving hyperglycemia, on a second portion of the supernatant (samples taken between 90 and 180 min only), 14C-labeled metabolites other than glucose were removed by ion-exchange chromatography (26). 3H and 14C disintegrations/min were determined using liquid scintillation counting (LS6500, Beckman Instruments, Fullerton, CA). 3H and 14C counts associated with liver and skeletal muscle glycogen were determined on aliquots from the extracts used to determine tissue glycogen concentration as described by Chan and Exton (2).

Metabolites, hormones, and tissue glycogen concentrations. Plasma glucose was determined using a Beckman glucose analyzer (glucose oxidase method; Glucose Analyzer II, Fullerton, CA). Insulin (Linco Research, St. Charles, MO) and glucagon (Linco) were determined by radioimmunoassay. Liver and skeletal muscle glycogen were determined using the method described by Chan and Exton (2).

Liver enzyme activities. All liver enzyme activities were determined at room temperature on extracts from tissue frozen immediately after animal death. The method described by Hutson et al. (12) was used to determine phosphorylase a activity. Glycogen synthase a activity was determined using the filter paper method of Thomas et al. (40). Glucokinase (GK) and glucose-6-phosphatase (G6Pase) activity were determined from the same liver homogenate. After centrifugation, the supernatant was used to determine GK activity at glucose concentrations of 0.5, 7, and 18 mM using methods previously described (4). G6Pase activity was determined on the reconstituted pellet at glucose-6-phosphatase concentrations of 0.5, 2.5, and 10 mM as described by Nordlie and Arion (18).

Data Analysis and Calculations

Glucose kinetics. Rate of appearance (Rd) of glucose under basal, euglycemic-hyperinsulinemic, and hyperglycemic-hyperinsulinemic conditions was determined by isotope dilution (38). Under basal conditions, basal Rd was calculated from the rolling average of glc SA at t = 70, 80, and 90 min. During the euglycemic and hyperglycemic clamps, the endogenous rate of glucose appearance (endo Rd) was calculated from the rolling average of 3H glc SA at t = 160, 170, and 180 min. Negative values for glucose endo Rd were not observed in this study (8, 29). The exogenous GIR during both euglycemic and hyperglycemic clamps was calculated as the time-weighted average over the last 45 min. Percent suppression of glucose endo Rd under euglycemic and hyperglycemic conditions was calculated as the ratio of endogenous to basal glucose endo Rd.

Tracer-estimated glycogenesis under basal and hyperglycemic conditions. Aliquots of the extracts resulting from the determinations of liver and skeletal muscle glycogen content were used to estimate retention of 3H and 14C-glucose in glycogen. In basal studies, retention of 3H in glycogen was calculated from the ratio of [3H]glycogen and [3H]glc SA in the portal vein. Under basal conditions, 3H retention into glycogen reflects the extent of tracer incorporation into liver glycogen, in the absence of net glycogenesis. In RESULTS, this is referred to as tracer-estimated glycogenesis. Glycogenesis during the hyperglycemic period was calculated from the ratio of [14C]glycogen and the weighted mean for 14C glc SA in arterial plasma over the 90-min hyperglycemic period. In

RESULTS, this is referred to as tracer-estimated net glycogenesis, because under this condition, the liver is characterized by net glucose uptake (20). The ratio of [3H]glucose/[14C]glucose in liver glycogen (corrected for [3H]glycogen at the end of the basal period) relative to that in arterial plasma (time weighted over the entire 90 min of hyperglycemia) was used to estimate the relative contributions of the direct and indirect pathways to tracer-estimated net hepatic glycogenesis. A ratio of 1.0 would indicate that all of the glycogen retained during the experiment was formed via the direct pathway; the lower the ratio, the greater the contribution of the indirect pathway to net glycogenesis. This method underestimates the contribution of the indirect pathway in proportion to the dilution of 14C traversing the oxaloacetate pool (14). It also assumes that this dilution is not different across diet groups (11). With these caveats, the tracer-estimated rate of net glycogen synthesis from the indirect pathway was calculated as the product of the tracer-estimated rate of net glycogenesis and the fraction arising from the indirect pathway.

Data Analyses

Two-way ANOVA was used to determine the statistical significance of any diet-by-time interactions, with contrasts used to detect any significant differences among groups. When one-way ANOVA in combination with necessary multiple-comparison tests (41) were used to detect differences across groups, interpretations were the same as those made using linear contrasts. Because the results from the one-way ANOVA and subsequent multiple comparisons offer more information to the reader, data are reported using these statistics. For glucose, insulin, and glucagon, repeated-measures ANOVA was used to detect any differences with respect to time. Significance was set at P≤ 0.05 for all comparisons. All data are reported as means ± SE. Statistical comparisons were not made in basal studies.

RESULTS

Basal Studies

Energy intake, fat pad weight, body weight gain, and body weight. Energy intake (cumulative pooled intake) averaged 103.7 ± 1.7 kcal at 1 wk and 519.9 ± 10.8 kcal at 5 wk. Fat pad weight (sum of epididymal, retroperitoneal, and mesenteric) was 15.9 ± 1.0 g in 1 wk and 31.2 ± 2.8 g in 5 wk. Body weight gain (cumulative weight gain) averaged 37.2 ± 2.9 g over 1 wk and 175.4 ± 8.3 g over 5 wk. Body weight on the day of study was 346 ± 7 g in 1 wk and 447 ± 11 g in 5 wk.

Plasma glucose, tissue glycogen, tracer-estimated glycogenesis, and hepatic enzyme activities. Fasting glucose concentrations, tissue glycogen content, and tracer-estimated glycogenesis are reported in Table 2. Activities for glycogen synthase a, glycogen phosphorlyase a, GK, and G6Pase are reported in Table 3.

Basal and Euglycemic Clamp Studies

Energy intake, fat pad weight, body weight gain, and body weight. There were no significant differences among 1- or 5-wk rats throughout the experimental diet-feeding period in energy intake (cumulative pooled intake: 102.9 ± 3.6 kcal at 1 wk; 514.9 ± 12.5 kcal at 5 wk), fat pad weight (sum of epididymal, retroperito-
neal, and mesenteric: 17.7 ± 2.3 g at 1 wk; 36.2 ± 3.1 g at 5 wk), body weight gain (cumulative weight gain: 34.9 ± 3.7 g at 1 wk; 174.9 ± 8.4 g at 5 wk), or body weight on the day of study (343 ± 8 g at 1 wk; 470 ± 13 g at 5 wk).

**Basal and clamp glucose and insulin concentrations.** Basal glucose (mg/dl) and insulin (ng/ml) concentrations were not significantly different among diet groups and averaged: basal glucose, HFD1, 122 ± 7; HSD1, 121 ± 3; STD1, 125 ± 4; HFD5, 124 ± 6; HSD5, 123 ± 3; STD5, 127 ± 4; basal insulin, HFD1, 2.3 ± 0.5; HSD1, 2.2 ± 0.5; STD1, 1.8 ± 0.4; HFD5, 2.6 ± 0.5; HSD5, 2.5 ± 0.5; STD5, 2.1 ± 0.5. Likewise, glucose and insulin concentrations during the euglycemic-hyperinsulinemic clamps were not significantly different among groups and averaged: clamp glucose (mg/dl), clamp insulin (ng/ml).

<table>
<thead>
<tr>
<th>Table 3. Hepatic enzyme activities at the end of basal studies</th>
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<tr>
<td></td>
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<tr>
<td>1 wk</td>
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<td>n = 3</td>
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<tr>
<td>Glycogen synthase a</td>
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<td>Glycogen phosphorylase a</td>
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<td>Glucokinase (18 mM glucose)</td>
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<td>Glucose-6-phosphatase (10 mM G6P)</td>
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<td>5 wk</td>
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<td>n = 4</td>
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<tr>
<td>Glycogen synthase a</td>
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<td>Glycogen phosphorylase a</td>
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<tr>
<td>Glucokinase (18 mM glucose)</td>
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<td>Glucose-6-phosphatase (10 mM G6P)</td>
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Values are means ± SE. Glycogen synthase and glucokinase and glucose-6-phosphatase represent maximal activities.
basal period in 1- and 5-wk rats, respectively (Fig. 1A). Plasma insulin at the end of the 90-min basal period was $2.7 \pm 0.4$ ng/ml (pooled average) for 1-wk rats and $2.2 \pm 0.3$ ng/ml for 5-wk rats, with no significant effect of diet (Fig. 1B). Likewise, there were no differences among diet groups in plasma glucagon at the end of the basal period (pooled average: $81.8 \pm 2.3$ ng/l at 1 wk; $81.8 \pm 2.4$ ng/l at 5 wk; Fig. 1C).

**Glucose kinetics under basal conditions.** Steady state was achieved over the last 20 min of the basal period for $^3$H glc SA (Fig. 2A). Basal R as (mg·kg$^{-1}$·min$^{-1}$) were not significantly different among 1- and 5-wk rats (pooled average: $15.7 \pm 1.4$ at 1 wk; $18.5 \pm 2.6$ at 5 wk; Fig. 3).

**Plasma substrate and hormone concentrations and glucose kinetics with hyperglycemia.** Glucose concentrations throughout the 90-min period of hyperglycemia were not significantly different among diet groups at any time point (Fig. 1A) and averaged $172 \pm 2$ mg/dl over the last 45 min of hyperglycemia for both 1- and 5-wk rats. The coefficient of variation for glucose concentrations averaged $5.1 \pm 0.3\%$. $^3$H and $^{14}$C glc SAs are shown in Fig. 2, A and B, respectively. GIR over the last 45 min of the hyperglycemic period was not significantly different among diet groups (pooled average: $6.4 \pm 0.8$ mg·kg$^{-1}$·min$^{-1}$ at 1 wk; $6.2 \pm 0.7$ mg·kg$^{-1}$·min$^{-1}$ at 5 wk; Fig. 3). Over the last 20 min of hyperglycemia, endo R as averaged $8.3 \pm 1.3$ mg·

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**Fig. 1.** Data are means $\pm$ SE; $n$ = 5–7/group for glucose (A) and glucagon (C) and $n$ = 5–6/group for insulin (B). HFD, high-fat diet; HSD, high-sucrose diet; STD, low-fat, high-starch diet.
kg\(^{-1}\)·min\(^{-1}\) for 1-wk rats and 6.3 ± 0.8 mg·kg\(^{-1}\)·min\(^{-1}\) for 5-wk rats with no significant effect of diet (Fig. 3). Suppression of glucose R\(_a\) with hyperglycemia averaged 44.4 ± 7.8% for 1-wk rats and 63.2 ± 4.3% for 5-wk rats and was not significantly different among diet groups (Fig. 3). There were no significant differences in plasma insulin or glucagon concentrations among diet groups at either \(t = 135\) or 180 during the hyperglycemic period (Fig. 1, B and C).

There was no significant accumulation of glucose or radioactivity in urine following the hyperglycemic period in any of the groups (data not shown).

Liver glycogen, tracer-estimated net hepatic glycogenesis, and enzyme activities. There were no significant differences among diet groups for liver glycogen content (pooled average: 4.5 ± 0.4 mg/g at 1 wk; 6.0 ± 0.7 mg/g at 5 wk), tracer-estimated net hepatic glycogenesis (7.8 ± 1.3 μg·g liver\(^{-1}\)·min\(^{-1}\) at 1 wk; 10.5 ± 2.2 μg·g liver\(^{-1}\)·min\(^{-1}\) at 5 wk).
μg·g liver$^{-1}$·min$^{-1}$ at 5 wk), percentage of indirect pathway glycogenesis ($52.8 \pm 6.7\%$ at 1 wk; $53.2 \pm 8.0\%$ at 5 wk), and net indirect pathway glycogenesis ($3.7 \pm 0.9 \mu g$ liver$^{-1}$·min$^{-1}$ at 1 wk; $3.4 \pm 0.9 \mu g$ liver$^{-1}$·min$^{-1}$ at 5 wk; Fig. 4). Maximal activities of glycogen synthase a, glycogen phosphorylase a, GK (at 18 mM glucose), and G6Pase (at 10 mM glucose-6-phosphate) were not significantly different among diet groups (Fig. 5).

Gastrocnemius glycogen and tracer-estimated net glycogenesis. There were no significant differences among diet groups in gastrocnemias glycogen concentration (pooled average: $3.4 \pm 0.5 \mu g/g$ at 1 wk; $3.3 \pm 0.4 \mu g/g$ at 5 wk) or tracer-estimated net glycogenesis (pooled average: $1.0 \pm 0.3 \mu g$ muscle$^{-1}$·min$^{-1}$ at 1 wk; $1.6 \pm 0.3 \mu g$ muscle$^{-1}$·min$^{-1}$ at 5 wk; Fig. 6).

DISCUSSION

Similar to previous studies (22, 24, 25), 1 wk of HFD and HSD feeding resulted in reduced insulin suppression of glucose Ra, or hepatic insulin resistance, under euglycemic, hyperinsulinemic conditions. Five weeks of HFD and HSD feeding produced both hepatic and peripheral insulin resistance. However, under hyperglycemic, hyperinsulinemic conditions, suppression of glucose Ra and stimulation of glucose disappearance were not reduced in HFD and HSD. In the present study, the effects of HFD and HSD feeding on liver glycogen synthesis were also investigated, because this process is both regulated by insulin and involves the gluconeogenic pathway (i.e., the indirect pathway) (16, 20, 30, 34). The results suggest that under hyperglycemic and hyperinsulinemic conditions, neither net hepatic glycogenesis nor the contribution of the indirect pathway to glycogen synthesis was significantly influenced by HFD or HSD.

These findings suggest that hyperglycemia compensated for HFD and HSD diet-induced insulin resistance. Under euglycemic and hyperinsulinemic conditions, HFD and HSD reduced 1) the GIR required to maintain glycemia, 2) suppression of endogenous glucose production, and 3) stimulation of glucose disappearance (5 wk only). In contrast, when hyperglycemia was induced in the presence of a similar level of hyperinsulinemia, GIRs, suppression of endogenous glucose production, and stimulation of glucose disappearance were not significantly different among HFD, HSD, and STD controls. In addition, the apparent contribution of the indirect pathway to hepatic glycogen synthesis was not significantly different among groups. Thus HFD and HSD diet-induced changes in gluconeogenic capacity (23, 27) do not appear to influence the contribution of gluconeogenic flux to liver glycogen formation under hyperglycemic conditions.

The ability of hyperglycemia to compensate for insulin resistance has been observed in humans. Insulin-resistant, normoglycemic first-degree relatives of patients with type 2 diabetes mellitus are characterized by increased glucose-mediated glucose disposal into extrahepatic tissues and increased glucose-mediated suppression of endogenous glucose production (10). Thus maintenance of fasting normoglycemia in the...
prediabetic state in humans is achieved, in part, through enhanced glucose effectiveness. It is likely that enhanced glucose effectiveness results from adaptations in glucose transport and metabolism. For example, recent studies have demonstrated that phosphoenolpyruvate carboxykinase gene expression can be suppressed by glucose metabolism (33). Hyperglycemia per se can also increase hepatic glycogen synthesis and increase the contribution of the direct pathway to total glycogen synthesis in rats (36).

In the present study, fasting hyperinsulinemia was not observed in either HFD or HSD groups after 1 or 5 wk. However, both groups of rats were clearly insulin resistant based on the euglycemic, hyperinsulinemic clamp technique. Thus, unlike the human, fasting hyperinsulinemia does not appear to be a consistent marker for insulin resistance in the rat. It is possible that maintenance of normoglycemia following exposure to HSD and HFD is achieved, in part, via changes in glucose effectiveness.

We have recently reported that in isolated hepatocytes, G6Pase activity is significantly increased in both HSD and HFD compared with STD (1). Although not evaluated statistically, basal G6Pase activities presented in Table 3 support these findings. However, in liver samples taken following the hyperglycemic, hyperinsulinemic period, these differences were no longer apparent. Normalization of hepatic G6Pase activity in HSD and HFD following the hyperglycemic, hyperinsulinemic period is consistent with the hypothesis that...
glucose effectiveness was increased in these two diet groups. The lack of differences among diet groups following the hyperglycemic, hyperinsulinemic period for all of the enzymes measured is also consistent with the tracer-estimated rates of endogenous glucose production and glycogen synthesis in the liver. It is important to note that any potential differences in enzyme responses may have been obscured by the 90-min glucose infusion period. For example, stimulation of glycogen synthase a has been observed after 15 min of exposure to hyperglycemia, whereas longer exposure resulted in activities that were not significantly different from baseline values (21).

The experimental protocol used to promote liver glucose uptake included a 90-min period of constant hyperglycemia and hyperinsulinemia and the delivery of glucose via a peripheral vein. In contrast, the postprandial setting is characterized by a dynamic rise and fall in glucose and insulin concentrations and the presence of a portal signal (elevation in the portal vein glucose concentration relative to the arterial glucose concentration) (9, 20). Because all groups were studied under similar conditions, these differences should not impact on the conclusions of this study. However, it would be predicted that the ability of hyperglycemia to compensate for insulin resistance is enhanced in the presence of constant and prolonged hyperglycemia.

The rates of endogenous glucose production we report during the hyperglycemic period may be underestimated. The hyperglycemic period, where both [6-3H] and [6,14C]glucose were infused, was preceded by a 90-min basal period during which [6-3H]glucose was also infused. During the basal period, 3H was retained in the liver glycogen pool. Thus, during the hyperglycemic period, hepatic glycogenolysis would result in the release of labeled glucose. We do not believe that this resulted in a large underestimate of glucose appearance because the specific activity of glycogen at the end of the basal period was only ~3% of the plasma glu SA. In addition, the amount of label in liver glycogen at the end of the basal period was not significantly different among groups.

Suppression of endogenous glucose production, whole body glucose disposal, and liver and skeletal muscle glycogen synthesis were not impaired under hyperglycemic, hyperinsulinemic conditions in rats following 1 or 5 wk on HFD or HSD. These diets impair insulin suppression of glucose production and insulin stimulation of glucose disposal and muscle glycogen synthesis under euglycemic, hyperinsulinemic conditions. Therefore, these data suggest that hyperglycemia compensates for diet-induced impairments in insulin action on glucose metabolism. In addition, although these diets increase the contribution of gluconeogenesis to glucose production under basal conditions, they do not appear to increase the contribution of the indirect pathway to liver glycogen synthesis under hyperglycemic conditions. Studies are underway to determine the mechanisms that contribute to changes in glucose effectiveness and increased G6Pase activity following exposure to HFD and HSD.

**Perspectives**

The liver has a central role in the disposal of dietary nutrients. This is due both to its anatomic location and its ability to remove a large variety of nutrients. In this context, the liver can be considered a dietary energy buffer. It would appear that when the macronutrient composition of the diet is changed, there are compensatory short- and long-term adjustments made by the liver. In the short term, the contribution of the liver to the removal of dietary nutrients may be increased. This is certainly the case for dietary sucrose, where the presence of fructose stimulates glucose uptake by the liver. These short-term adjustments lead to long-term adaptations that include changes in gluconeogenic capacity and insulin action. The ability of hyperglycemia to compensate for some of these long-term adaptations provides a mechanism to reduce the magnitude of postprandial hyperglycemia. The signals that ultimately lead to these short- and long-term adjustments in liver glucose metabolism are an important area for future study and will certainly provide important clues in the quest to understand hepatic impairments in both prediabetic and diabetic states.

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**REFERENCES**


