Insulin resistance of muscle glucose transport in male and female rats fed a high-sucrose diet

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Kim, Jong-Yeon, Lorraine A. Nolte, Polly A. Hansen, Dong-Ho Han, Kentaro Kawanaka, and John O. Holloszy. Insulin resistance of muscle glucose transport in male and female rats fed a high-sucrose diet. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R665–R672, 1999.—It has been reported that, unlike high-fat diets, high-sucrose diets cause insulin resistance in the absence of an increase in visceral fat and that the insulin resistance develops only in male rats. This study was done to 1) determine if isolated muscles of rats fed a high-sucrose diet are resistant to stimulation of glucose transport when studied in vitro and 2) obtain information regarding how the effects of high-sucrose and high-fat diets on muscle insulin resistance differ. We found that, compared with rat chow, semipurified high-sucrose and high-starch diets both caused increased visceral fat accumulation and insulin resistance of skeletal muscle glucose transport. Insulin responsiveness of 2-deoxyglucose (2-DG) transport measured in epitrochlearis and soleus muscles in vitro was decreased ~40% (P < 0.01) in both male and female rats fed a high-sucrose compared with a chow diet. The high-sucrose diet also caused resistance of muscle glucose transport to stimulation by contractions. There was a highly significant negative correlation between stimulated muscle 2-DG transport and visceral fat mass. In view of these results, the differences in insulin action in vivo observed by others in rats fed isocaloric high-sucrose and high-starch diets must be due to additional, specific effects of sucrose that do not carry over in muscles studied in vitro. We conclude that, compared with rat chow, semipurified high-sucrose and high-cornstarch diets, like high-fat diets, cause increased visceral fat accumulation and severe resistance of skeletal muscle glucose transport to stimulation by insulin and contractions.

2-deoxyglucose; muscle contractions; high-starch diet

THE ABDOMINAL OR VISCERAL obesity syndrome, which is the major cause of type 2 diabetes, is characterized by central/vvisceral obesity, insulin resistance, hyperinsulinemia, impaired or diabetic glucose tolerance, and plasma lipid and lipoprotein abnormalities (3, 4, 22). Rodents that are fed a high-fat diet rapidly develop impaired intracellular glucose metabolism (21), visceral obesity, whole body and skeletal muscle insulin resistance, hyperinsulinemia, hyperglycemia, hyperlipidemia, and, if the high-fat diet is continued sufficiently long, diabetes in genetically susceptible animals (12, 13, 20, 23, 27, 35, 40). On the basis of these findings, it has been suggested that high-fat diet-fed rodents develop a syndrome that is the equivalent of, and that can serve as a model for the development of, the abdominal obesity syndrome in humans (5, 13, 38).

Feeding diets high in sucrose to rats also results in rapid development of insulin resistance of a magnitude similar to that induced by high-fat diets (28–30, 37). It has been reported that, in contrast to the effects of a high-fat diet, sucrose feeding results in insulin resistance without causing visceral or generalized obesity (28–30, 37) and that this effect occurs only in male, not in female, rats (19). The sucrose-induced insulin resistance, evaluated using the euglycemic clamp procedure, was found to involve the liver, with increased hepatic glucose production over a range of insulin concentrations, and skeletal muscle, with decreased peripheral glucose utilization (28–30, 37). The muscle insulin resistance induced by a high-sucrose (HSu) diet has been attributed to an elevation of plasma triglyceride concentration (29).

Skeletal muscle insulin resistance induced by feeding rats a high-fat diet is demonstrable in muscles that have been incubated in vitro for 1–2 h, providing evidence for adaptations intrinsic to the muscle, i.e., independent of the acute effects of plasma substrate levels (13, 15). In view of the evidence that an HSu diet mediates insulin resistance by different mechanisms than a high-fat diet, the present study was undertaken as a preliminary characterization of the effects of sucrose feeding on stimulated glucose transport in isolated muscles.

In our initial experiment, we found that, compared with a rat chow diet, HSu and high-cornstarch (HSt) diets caused muscle insulin resistance and increased visceral fat accumulation. This finding was unexpected, because in previous studies in which HSu diets caused insulin resistance, the control rats were pair fed an isocaloric HSt diet (19, 28–30, 37). We therefore did additional studies to characterize the effect of the HSu diet on muscle glucose transport and to obtain preliminary information regarding whether HSu diet-fed rats can, like fat-fed rats, be used to evaluate the relationship between visceral fat accumulation and muscle insulin resistance.

MATERIALS AND METHODS

Materials

2-[1,2-3H]deoxy-D-glucose (2-DG) was obtained from American Radiolabeled Chemicals (St. Louis, MO), and d-[1-14C]mannitol was obtained from NEN Life Science Products (Boston, MA). Insulin (Novolin) was purchased from Novo Nordisk (Princeton, NJ). Polyclonal antiserum specific for the GLUT-4 glucose transporter was the generous gift of Dr. Mike Mueckler (Washington University, St. Louis, MO). Horseradish peroxidase-conjugated donkey anti-rabbit IgG was pur-

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chased from Jackson ImmunoResearch Laboratories (West Grove, PA). Reagents for enhanced chemiluminescence were obtained from Amersham (Arlington Heights, IL). All other reagents were obtained from Sigma (St. Louis, MO).

**Treatment of Animals**

Male (−50 g) and female (−50 g) Wistar rats were obtained from Charles River and placed on either an HSu, HSt, or rat chow diet. The HSu diet contained, in grams per 100 grams, 65 sucrose, 20 casein, 0.3 methionine, 5 corn oil, 5 cellulose, 1 vitamin mixture, 3.5 mineral mixture, and 0.2 choline bitartrate. The HSt diet had the same composition as the HSu diet except that 50 g cornstarch and 15 g maltodextrin were substituted for sucrose. These diets are the same as those used by Pagliassotti et al. (see Ref. 30 for details) and were obtained from Research Diets (New Brunswick, NJ). The rat chow, Constant-Formula Purina Rodent Chow no. 5001, was obtained from Purina Mills (St. Louis, MO); it contained, in percent of total calories, 58.9 carbohydrate, 12.4 fat, and 28.7 protein. The energy content of the HSu and HSt diets was 4.1 kcal/g, whereas that of the rat chow was 3.3 kcal/g. The rats were provided the diets and water ad libitum. This research was approved by the Animals Studies Committee of Washington University.

**Tissue Collection**

Food was removed after 6:00 PM the day before the experiment. Rats were anesthetized by an intraperitoneal injection of pentobarbital sodium (50 mg/kg body wt), blood samples for measurement of plasma glucose, insulin, triglyceride, and leptin were drawn from a tail vein, and then the epididymal or ovarian, mesenteric, and retroperitoneal fat pads were removed and weighed. After the muscle dissection was completed, the abdominal cavity was opened and the epididymal or ovarian, mesenteric, and retroperitoneal fat pads were removed and weighed.

**Muscle Incubations: Effects of Insulin**

To allow recovery from the dissection and splitting procedures, muscles were incubated for 30 min at 35°C in a shaking incubator in 2 ml of oxygenated Krebs-Henseleit buffer (KHB) supplemented with 8 mM glucose, 32 mM mannitol, and 0.1% BSA. After the 30-min recovery period, epimysial muscles and soleus strips were incubated for 60 min at 35°C in 2 ml of oxygenated KHB containing 8 mM glucose, 32 mM mannitol, and 0.1% BSA in the presence or absence of a maximally effective concentration of insulin (2 mU/ml) before measurement of 2-DG transport activity.

**Muscle Stimulation**

For experiments involving activation of glucose transport by contractile activity, one epitrochlearis muscle of each rat was stimulated indirectly via the ulnar nerve, as described previously (11). Stimulated muscles and contralateral control muscles were excised after the last stimulation period. Muscles were then transferred to 2 ml oxygenated KHB containing 40 mM mannitol and 0.1% BSA at 30°C and incubated for 10 min to wash glucose out of the extracellular space before measurement of 2-DG transport activity.

**Measurement of 2-DG Transport Activity**

Muscle 2-DG transport activity was measured using 2-DG, as described previously (14). After incubation with insulin or stimulation of contraction, muscles were incubated at 30°C for 20 min in 2 ml KHB containing 4 mM 2-[1,2-3H]deoxyglucose (1.5 µCi/ml), 36 mM [6-3H]mannitol (0.2 µCi/ml), 0.1% BSA, and insulin if it was present in the previous incubation. Extracellular space and intracellular 2-DG concentration were determined as previously described (39).

**Measurement of Immunoreactive GLUT-4 Protein**

Muscle GLUT-4 glucose transporter content was determined by Western blotting as described previously (16), with a rabbit polyclonal antibody directed against the COOH terminus of GLUT-4 followed by horseradish peroxidase-conjugated anti-rabbit IgG. Antibody-bound transporter protein was visualized with enhanced chemiluminescence according to the manufacturer’s specifications. Protein bands were quantitated using densitometry.

**Analytic Procedures**

Plasma (from tail vein blood) glucose concentration was determined using the glucose oxidase method with a Beckman glucose analyzer II (Beckman Instruments, Fullerton, CA). Plasma insulin and leptin were measured by radioimmunoassay. Serum triglyceride concentration was measured using a kit obtained from Sigma. Muscle triglyceride concentration was determined by extracting total lipids from clamp-frozen muscle samples with chloroform-methanol (2:1, vol/vol) as described by Flodh et al. (9), separating the chloroform and methanol-water phases, removing phospholipids, and further processing the sample using Frayn and Maycock’s (10) modification of the method of Denton and Randle (8). Triglycerides were then quantified spectrophotometrically as glycerol using an enzymatic assay kit (Sigma). Muscle glycerogen was measured using the amyloglucosidase method (32).

**Statistical Analysis**

Values are expressed as means ± SE. The significance of difference between two groups was evaluated using Student’s t-test. For multiple comparisons, a one-way ANOVA was used. When ANOVA showed significant differences, post hoc analysis was performed with the Newman-Keuls multiple range test. Nonlinear regression analyses were performed using Sigma Plot 4.0 (SPSS Science, Chicago, IL).

**RESULTS**

**Effects of HSu and HSt Diets on Muscle Glucose Transport Activity**

The insulin responsiveness of the glucose transport process was measured in epitrochlearis and soleus muscles in vitro using 2-DG. The epitrochlearis is a fast-twitch muscle that contains ~76% type IIb fibers, 12% type IIa fibers, and 12% type I fibers (34); the soleus is a slow-twitch muscle that consists of ~87% type I fibers and 13% type IIa fibers (1). As shown in Fig. 1, the increase in 2-DG transport induced by a maximally effective insulin stimulus was significantly reduced in epitrochlearis muscles of male rats fed either the HSu or the HSt diet for 4 wk compared with those fed rat chow. Insulin-stimulated
glucose transport was similarly decreased in soleus muscles of the HSu and the HSt groups (Fig. 1).

Effect of HSu Diet on Insulin-Stimulated Muscle Glucose Transport in Female Rats

In view of the evidence that an HSu (compared with an HST) diet does not cause whole body insulin resistance in female rats (19), we compared maximally insulin-stimulated glucose transport activity in muscles of female rats fed either the HSu or the chow diet. As shown in Fig. 2, the increases in 2-DG transport induced by insulin were also significantly blunted in the epitrochlearis and soleus muscles of female rats fed the HSu diet for 4 wk compared with controls fed the chow diet.

Contraction-Stimulated Glucose Transport Activity

As shown in Fig. 3, the increase in 2-DG transport induced by in situ electrical stimulation of contractile activity was significantly smaller, by ~46%, in epitrochlearis muscles of the HSu-fed group than in those of the chow-fed controls. Thus contraction-stimulated glucose transport activity was decreased to about the same extent as insulin-stimulated transport in response to the HSu diet.
TABLE 1. Muscle triglyceride and glycogen concentrations

<table>
<thead>
<tr>
<th>Diet Group</th>
<th>Muscle triglyceride</th>
<th>Glycogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soleus</td>
<td>7.2 ± 0.7 (6)</td>
<td>1.8 ± 0.1 (5)</td>
</tr>
<tr>
<td>Gastrocnemius</td>
<td>1.6 ± 0.1 (4)</td>
<td>2.7 ± 0.2* (6)</td>
</tr>
<tr>
<td>Female</td>
<td>6.2 ± 0.5 (6)</td>
<td>8.0 ± 0.7 (7)</td>
</tr>
<tr>
<td>Soleus</td>
<td>1.8 ± 0.4 (5)</td>
<td>2.9 ± 0.4* (6)</td>
</tr>
<tr>
<td>Gastrocnemius</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muscle glycogen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>11.3 ± 1.0 (6)</td>
<td>10.8 ± 0.8 (5)</td>
</tr>
<tr>
<td>Epitrochlearis</td>
<td>11.8 ± 1.0 (5)</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE in µmol/g wet wt for number of muscles shown in parentheses. *P < 0.05 vs. chow-fed group.
There was no significant correlation between insulin-stimulated 2-DG transport in the soleus muscle and soleus muscle triglyceride concentration in male (\(r = 0.432, P < 0.141\)) or in female rats (\(r = -0.364, P < 0.221\)).

**Plasma Triglyceride Concentration**

As shown in Table 3, plasma triglyceride concentration was higher in the male rats on the HSu diet after both 4 and 8 wk on the diet and in the female HSu diet group after 4 wk than in the comparable chow-fed groups. Plasma triglycerides in the HSt group were intermediate between the HSu and chow group values at 4 wk.

**Plasma Leptin Concentration**

Plasma leptin levels were significantly higher in both the male and female rats on the HSu diet and also in the male rats on the HSt diet than in their controls on the chow diet (Table 3).

**Effect of Decreased Food Intake in Rats on HSu Diet**

To try to distinguish between a specific effect of sucrose and an effect of increased visceral fat accumulation, we did an experiment in which ad libitum-fed rats on the HSu and chow diets were compared with animals given \(~75\%\) as much of the HSu diet each day as was eaten by the ad libitum-fed HSu diet group. As shown in Table 4, the rats on a restricted intake of the HSu diet had significantly lower body weights and total visceral fat weights than either the HSu or chow ad libitum-fed groups.

As shown in Fig. 6, the caloric restriction completely protected against the insulin resistance of muscle glucose transport induced by an HSu diet. It also protected against the increase in plasma triglycerides (Table 4).

**DISCUSSION**

It is well established that when the euglycemic, hyperinsulinemic clamp procedure is used to compare insulin action in rats fed either HSu or HSt diets, the HSu-fed rats are more resistant to insulin than the starch-fed animals (28–30, 37). A major effect of an HSu

<table>
<thead>
<tr>
<th>Time on Diet, wk</th>
<th>Diet Group</th>
<th>Triglycerides, mg/dl</th>
<th>Leptin, ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male rats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Chow</td>
<td>68 ± 4 (25)</td>
<td>0.82 ± 0.13 (20)</td>
</tr>
<tr>
<td></td>
<td>HSu</td>
<td>79 ± 7† (23)</td>
<td>1.28 ± 0.27 (5)</td>
</tr>
<tr>
<td></td>
<td>HSt</td>
<td>97 ± 11‡ (6)</td>
<td>3.28 ± 1.02* (5)</td>
</tr>
<tr>
<td>8</td>
<td>Chow</td>
<td>60 ± 6 (6)</td>
<td>0.002 ± 0.24 (5)</td>
</tr>
<tr>
<td></td>
<td>HSu</td>
<td>62 ± 9 (6)</td>
<td>1.28 ± 0.27 (5)</td>
</tr>
<tr>
<td></td>
<td>HSt</td>
<td>79 ± 13‡ (6)</td>
<td>3.28 ± 1.02* (5)</td>
</tr>
<tr>
<td>Female rats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Chow</td>
<td>45 ± 6 (8)</td>
<td>0.05 ± 0.08 (8)</td>
</tr>
<tr>
<td></td>
<td>HSu</td>
<td>55 ± 9† (8)</td>
<td>0.63 ± 0.08 (8)</td>
</tr>
<tr>
<td></td>
<td>HSt</td>
<td>73 ± 5† (8)</td>
<td>0.90 ± 0.06† (8)</td>
</tr>
</tbody>
</table>

Values are means ± SE for number of rats shown in parentheses. *P < 0.05, †P < 0.01, ‡P < 0.001 vs. Chow-fed group.
diet is on the liver, which becomes markedly resistant to the suppression of glucose production by insulin (28–30, 37). There is also a reduction in peripheral glucose uptake (28–30, 37). Podolin et al. (33) found a marked decrease in insulin-stimulated muscle 2-DG uptake in HSu-fed compared with HSt-fed rats given a bolus injection of 2-DG during a hyperinsulinemic clamp. In contrast, Storlein et al. (37), using very similar diets and methodology, found little or no difference in skeletal muscle 2-DG accumulation between sucrose- and HSt-fed animals. Both groups found that, as in the present study, there were no differences in body weight gain or in visceral or total fat accumulation between rats fed isocaloric HSu and HSt diets (28–30, 37). In addition, Pagliassotti’s group (19) found that female rats do not develop sucrose-induced insulin resistance.

In view of these findings, it appeared that HSu and high-fat diets induce insulin resistance by quite different mechanisms, because high-fat feeding results in a rapid increase in visceral fat (13, 23, 24) and causes insulin resistance not only in male but also in female rodents (Refs. 20 and 25 and P. A. Hansen and J. O. Holloszy, unpublished findings). However, the results of the present study require some modification of this interpretation. In keeping with the results of the previous studies, we found that rats fed HSu or HSt diets had similar body weight gains and visceral fat accumulation; this is not surprising because these semipurified diets have the same caloric density. However, both the HSu- and the HSt-fed rats accumulated 50% more visceral fat in 4 wk than did chow-fed controls. This difference is likely due to the lower caloric density of the chow diet, which resulted in a ~10% lower average energy intake. Furthermore, muscles of both the HSu diet- and HSt-fed rats were severely insulin resistant compared with those of the chow-fed animals.

Although our results provide evidence that HSu and HSt diets have similar effects on visceral fat accumulation and induce similar degrees of insulin resistance of glucose transport when muscles are studied in vitro, it seems clear from previous studies that an HSu diet has additional effects. In these studies in which rats were pair fed isocaloric HSu and HSt diets, the HSu-fed male (but not female) rats had significantly more hepatic and peripheral insulin resistance than the starch-fed animals when evaluated with the hyperinsulinemic clamp procedure (28–30, 37). This difference between the results obtained in vivo and in vitro provides evidence that, in addition to causing increased visceral fat accumulation and adaptations in muscle that result in a blunting of insulin-stimulated glucose transport, HSu diets have additional effects (only) in male rats. Because HSu and HSt diets induce a similar degree of insulin resistance in muscle that is evident in vitro, it seems reasonable that an additional inhibitory effect induced by an HSu diet on glucose transport must be mediated by a humoral factor(s). It has been hypothesized that the peripheral insulin resistance induced by sucrose feeding is mediated by elevated plasma triglycerides (29), but experimental support for this hypothesis is lacking.

It is well documented in studies on humans that abdominal or visceral obesity is associated with a metabolic syndrome that includes insulin resistance, hyperinsulinemia, impaired glucose tolerance that fre-
quenty progresses to type 2 diabetes, and plasma lipid and lipoprotein abnormalities (3, 4, 22). Feeding rats a high-fat diet results in what appears to be the rodent equivalent of the visceral obesity syndrome, with development over a ~6-mo period of gross visceral and whole body obesity, impaired or diabetic glucose tolerance, and lipid and lipoprotein abnormalities (13). Judging from the effects of 8 wk of the HSu diet in the present study, it seems likely that this syndrome would also occur in rats fed an HSu diet over a sufficiently long period. However, it is clear from the results of studies on rodents fed high-fat diets as well as from the present results that severe muscle insulin resistance develops within 4 wk (13, 20, 23, 35, 40), a time at which visceral fat accumulation is increased by ~50% (13, 40) but long before the development of gross obesity.

In this context, it is our working hypothesis that the metabolic-hormonal consequences of obesity are mediated by a strongly positive "visceral fat balance," i.e., a rapid accumulation of fat in visceral adipocytes, rather than by obesity per se. A number of lines of evidence support this hypothesis. Plasma insulin levels are elevated in rats fed high-fat diets for a few weeks, long before obesity develops (13). In the present study, both plasma insulin and leptin concentrations were significantly elevated after 4 wk on the semipurified high-carbohydrate diets, at which time body weight was minimally affected but visceral fat mass was increased by 50%. A rapid improvement in insulin action occurs in response to caloric restriction which results in negative fat balance with triglyceride hydrolysis and release of fatty acids from, instead of fat storage in, the fat cells, even when there is minimal weight loss (6, 7, 18). Of particular interest in this regard is a study by Barzilai et al. (2) showing that leptin administration selectively decreases visceral adiposity and markedly enhances insulin-stimulated glucose uptake during a euglycemic, hyperinsulinemic clamp.

It has been hypothesized that high-fat diets cause muscle insulin resistance by increasing skeletal muscle, rather than visceral adipocyte, triglyceride content (31, 36). The present finding that purified high-carbohydrate diets cause a similar degree of insulin resistance in the soleus muscle without a significant increase in triglyceride content argues against this hypothesis. On the other hand, the HSu diet and high-fat diet caused similar increases in visceral fat after 4 and 8 wk on the diets (Ref. 13 and Table 2), and there was an excellent inverse correlation between visceral fat weight and insulin- or contraction-stimulated glucose transport in muscle in the present study. Of particular interest in this regard is the finding that the HSu diet only caused insulin resistance when it resulted in positive energy balance and an increase in visceral fat. When the HSu diet was fed in restricted amounts, ~75% of ad libitum intake, visceral fat mass was reduced and muscle insulin responsiveness was normal.

Perspectives

Our study comparing the effects of feeding rats HSt, HSu, and rat chow diets on stimulated glucose transport measured in muscles in vitro, together with previous studies comparing the effects of HSu and HSt diets on insulin action in vivo, provides evidence for two separate sets of responses to sucrose feeding. As shown in the present study, responses that HSu and HSt diets have in common include a large increase in visceral fat accumulation and adaptations in muscle that result in resistance of glucose transport to stimulation by insulin or contractions in vitro. As shown in studies by others, responses mediated by an HSu, but not an HSt, diet and that occur only in male, not in female, rats include increases in liver and plasma triglyceride concentrations, severe hepatic insulin resistance, and an additional inhibitory effect on insulin-stimulated peripheral glucose uptake that is demonstrable in vivo.

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