Effects of fructose and glucose on plasma leptin, insulin, and insulin resistance in lean and VMH-lesioned obese rats

Asako Suga, Tsutomu Hirano, Haruaki Kageyama, Toshimasa Osaka, Yoshio Namba, Masatomi Tsuji, Masakazu Miura, Mitsuru Adachi, and Shuji Inoue. Effects of fructose and glucose on plasma leptin, insulin, and insulin resistance in lean and VMH-lesioned obese rats. Am J Physiol Endocrinol Metab 278: E677–E683, 2000.—To determine the influence of dietary fructose and glucose on circulating leptin levels in lean and obese rats, plasma leptin concentrations were measured in ventromedial hypothalamic (VMH)-lesioned obese and sham-operated lean rats fed either normal chow or fructose- or glucose-enriched diets (60% by calories) for 2 wk. Insulin resistance was evaluated by the steady-state plasma glucose method and intravenous glucose tolerance test. In lean rats, glucose-enriched diet significantly increased plasma leptin with enlarged parametrial fat pad, whereas neither leptin nor fat-pad weight was altered by fructose. Two weeks after the lesions, the rats fed normal chow had marked greater body weight gain, enlarged fat pads, and higher insulin and leptin compared with sham-operated rats. Despite a marked adiposity and hyperinsulinemia, insulin resistance was not increased in VMH-lesioned rats. Fructose brought about substantial insulin resistance and hyperinsulinemia in both lean and obese rats, whereas glucose led to enhanced insulin sensitivity. Leptin, body weight, and fat pad were not significantly altered by either fructose or glucose in the obese rats. These results suggest that dietary glucose stimulates leptin production by increasing adipose tissue or stimulating glucose metabolism in lean rats. Hyperleptinemia in VMH-lesioned rats is associated with both increased adiposity and hyperinsulinemia but not with insulin resistance. Dietary fructose does not alter leptin levels, although this sugar brings about hyperinsulinemia in the body, irrespective of subcutaneous or visceral obesity (3, 5, 6), suggesting that circulating leptin is an excellent indicator of adiposity in the entire body. Furthermore, it is important to know what factors influence plasma leptin levels to identify the role of circulating leptin in vivo. The weight-reduction action of leptin is thought to be mediated primarily by signal transduction through the leptin receptor in the hypothalamus (3). Bilateral lesions of the ventromedial nuclei in the hypothalamus (VMH) can produce obesity in rats, and the lesioned rats have been widely used as a representative animal model of obesity (2, 20). VMH-lesioned obese rats have a significant hyperphagia despite substantially increased leptin levels (7, 24), which is consistent with the results that a key target for the biological actions of leptin is destroyed by producing VMH lesions (12). It is also interesting to know whether leptin production is altered by dietary sugars in VMH-lesioned animals when leptin production is noticeably stimulated.

The present study was designed to examine the influence of dietary sugars on leptin production in vivo. The weight-reduction action of leptin is thought to be mediated primarily by signal transduction through the leptin receptor in the hypothalamus (3). Bilateral lesions of the ventromedial nuclei in the hypothalamus (VMH) can produce obesity in rats, and the lesioned rats have been widely used as a representative animal model of obesity (2, 20). VMH-lesioned obese rats have a significant hyperphagia despite substantially increased leptin levels (7, 24), which is consistent with the results that a key target for the biological actions of leptin is destroyed by producing VMH lesions (12). It is also interesting to know whether leptin production is altered by dietary sugars in VMH-lesioned animals when leptin production is noticeably stimulated. The present study was designed to examine the influence of dietary sugars on plasma leptin levels in a representative animal model of obesity (2, 20). VMH-lesioned obese rats have a significant hyperphagia despite substantially increased leptin levels (7, 24), which is consistent with the results that a key target for the biological actions of leptin is destroyed by producing VMH lesions (12). It is also interesting to know whether leptin production is altered by dietary sugars in VMH-lesioned animals when leptin production is noticeably stimulated.
when insulin resistance was not yet present (24). It is well known that dietary fructose but not glucose induces insulin resistance in rats (14, 26, 28). Therefore, it is of interest to explore the relationship between plasma leptin concentration and insulin resistance in lean and VMH-lesioned rats when their insulin secretion or insulin sensitivity is modified by dietary sugars.

**MATERIALS AND METHODS**

**Rats**

Female Sprague-Dawley rats weighing 240–280 g (Japan SCL, Hamamatsu, Japan) were kept in individual cages on a rotating 12:12-h light-dark cycle and given free access to food and water. The animals were anesthetized by inhalation of isoflurane (Forane, Dainabot, Osaka, Japan), and electrolytic bilateral VMH lesions were produced by the method previously described (11). Control animals received sham VMH lesions (no current passed through the electrode). Fructose- or glucose-fed groups of rats ingested a purified experimental diet (Oriental Food, Tokyo, Japan) that contained (as a percentage of calories) either 60% fructose or 60% glucose, 11% corn oil, and 29% animal protein. The chow-fed group was fed standard rat chow (Oriental Food), which contained 60% vegetable starch, 11% corn oil, and 29% animal protein. Food consumption was restricted in some VMH-lesioned rats to conduct pair feeding with control animals. These rats were given 15 g of chow/day, which is the amount usually consumed by sham-operated control rats. Fructose or glucose feedings were started immediately after the VMH operation. Food consumption of normal chow, fructose-enriched, or glucose-enriched diets was given for 2 wk. Food was withdrawn at 0900 on the day of the experiments, and all experiments were carried out after a 5-h fast (0900 to 1400). The parametrial fat pad was removed and weighed immediately after the kinetic studies.

Steady-state plasma glucose method for evaluation of insulin resistance. Insulin resistance in the whole body was assessed by the steady-state plasma glucose (SSPG) method originally developed by Reaven’s laboratory (27, 28) and modified by Harano et al. (9). Rats were anesthetized with pentobarbital sodium and then given a constant infusion of 0.5 µg/min, insulin (2.5 mU·kg⁻²·min⁻¹, Humulin R, Eli Lilly-Shionogi, Osaka, Japan) and somatostatin (0.5 µg/min, Sigma, St. Louis, MO) for 170 min through a cannula inserted into the right jugular vein. Blood samples were collected before the infusion was initiated and 150, 160, and 170 min after the infusion from a cannula inserted into the femoral vein. Under these conditions, endogenous insulin release was inhibited by somatostatin, and SSPG was maintained during the last 20 min of the infusion. The mean of 150-, 160-, and 170-min samples was used to determine the SSPG values. Because the SSPG response is a direct reflection of the efficiency of insulin-mediated glucose disposal, higher values imply proportionally greater insulin resistance.

Intravenous glucose tolerance test (IVGTT) involved a bolus injection via catheter of a 0.5 g/ml glucose solution at a dose of 1.0 g/kg body wt. Blood samples were collected before the injection and 5, 10, 30, and 60 min afterward. The blood was immediately centrifuged at 4°C, and plasma was stored at −20°C until assayed. Insulin area under the curve (AUCins) was calculated.

**Measurements**

Plasma glucose levels were determined by the glucose oxidase method (Glucose B-test, Wako Pure Pharmaceutical, Osaka, Japan). Immunoreactive insulin (IRI) concentrations were determined by a RIA kit (RI-13K, Linco Research, St. Charles, MO) standardized against rat insulin. Plasma leptin concentrations were determined by a RIA kit (RL-83K, Linco Research) specifically for determining rat leptin.

**Statistics**

Data are expressed as means ± SE. Statistical analyses were performed with the Stat View J-4.5 for Macintosh system. Statistical significance was assessed by one-way ANOVA, and P < 0.05 was accepted as a significant difference. The correlation coefficients between two parameters were determined by Pearson’s simple linear regression analysis.

**RESULTS**

Profiles of sham-operated and VMH-lesioned rats. Table 1 shows general and metabolic profiles of sham-operated and VMH-lesioned rats given normal chow, fructose-enriched, or glucose-enriched diet. The food intake of rats with VMH lesions fed normal chow was increased twofold compared with that of sham-operated rats. Fructose or glucose feeding slightly increased the amount of food intake in sham-operated rats, whereas this sugar feeding did not affect the amount of food intake in VMH-lesioned rats.

**Table 1. General profiles of sham-operated and VMH-lesioned rats given normal chow, fructose-enriched, or glucose-enriched diets**

<table>
<thead>
<tr>
<th></th>
<th>Normal Chow</th>
<th>Fructose</th>
<th>Glucose</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Sham</td>
<td>VMH</td>
<td>Sham</td>
</tr>
<tr>
<td>n</td>
<td>17</td>
<td>16</td>
<td>14</td>
</tr>
<tr>
<td>Initial BW, g</td>
<td>258 ± 3</td>
<td>270 ± 3a</td>
<td>253 ± 6a</td>
</tr>
<tr>
<td>Final BW, g</td>
<td>276 ± 4</td>
<td>344 ± 5a</td>
<td>262 ± 6a</td>
</tr>
<tr>
<td>BW gain, g/day</td>
<td>1.3 ± 0.2</td>
<td>5.3 ± 0.4a</td>
<td>0.6 ± 0.2ac</td>
</tr>
<tr>
<td>Parametrial fat-pad, g</td>
<td>3.0 ± 0.4</td>
<td>7.5 ± 0.6b</td>
<td>3.2 ± 1.4c</td>
</tr>
<tr>
<td>Food intake, g/day</td>
<td>13.7 ± 0.8</td>
<td>29.3 ± 9.3b</td>
<td>18.2 ± 5.8b, c</td>
</tr>
<tr>
<td>Plasma insulin, ng/ml</td>
<td>1.1 ± 0.1</td>
<td>2.5 ± 0.3a</td>
<td>2.1 ± 0.3a</td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>7.2 ± 0.5</td>
<td>8.0 ± 0.8</td>
<td>7.3 ± 0.4</td>
</tr>
</tbody>
</table>

General profiles of sham-operated and ventromedial hypothalamus (VMH)-lesioned rats given normal chow, fructose-enriched, or glucose-enriched diets for 2 wk. Blood was collected after 5-h fast. Data represent means ± SE; n = number of rats; BW, body weight. Statistical significance by one-way ANOVA at P < 0.05; a, vs. sham-operated rats fed normal chow; b, vs. VMH-lesioned rats fed normal chow; c, vs. sham-operated rats fed fructose-enriched diet; d, vs. VMH-lesioned rats fed fructose-enriched diet; e, vs. sham-operated rats fed glucose-enriched diet.
of food intake in VMH-lesioned rats. The VMH-lesioned rats gained body weight at fourfold higher rates and had twofold increased parametrial fat-pad weight compared with sham-operated rats. Fructose or glucose feeding tended to suppress body weight gain in sham-operated rats; however, these figures did not reach statistical significance. Parametrial fat-pad weight became significantly heavier by glucose feeding compared with chow or fructose feeding in lean rats. In VMH-lesioned rats, neither fructose nor glucose feeding affected body weight gain or fat-pad weight. Plasma glucose levels were not altered significantly after VMH lesions or dietary sugar, except that glucose feeding slightly decreased the plasma glucose level in VMH-lesioned rats.

Plasma levels of leptin. As depicted in Fig. 1, plasma leptin concentrations were increased sixfold in VMH-lesioned rats fed normal chow compared with sham-operated rats (19.7 ± 1.9 vs. 2.6 ± 0.3 ng/ml). Fructose feeding did not affect leptin levels in either sham-operated (3.2 ± 0.3 vs. 2.6 ± 0.3) or VMH-lesioned rats (16.4 ± 1.9 vs. 19.7 ± 1.9) compared with chow-fed counterparts. Dietary glucose increased plasma leptin levels 2.4-fold in sham-operated rats (6.3 ± 1.0 vs. 2.6 ± 0.3), whereas glucose feeding did not alter leptin levels in VMH-lesioned rats (21.3 ± 2.0 vs. 19.7 ± 1.9). Thus the magnitude of hyperleptinemia was comparable among chow, fructose, and glucose feeding in the obese animals. As depicted in Fig. 2, plasma leptin concentrations were highly correlated with body weight gain or parametrial fat-pad weight in all groups of animals.

Fig. 1. Plasma leptin concentrations in (A) sham-operated and (B) VMH-lesioned rats given normal chow, fructose-enriched, or glucose-enriched diets for 2 wk. Blood was collected after 5-h fast. Data represent means ± SE; nos. in bars, nos. of rats/group. Statistical significance by one-way ANOVA at P < 0.05: a, vs. sham-operated rats fed normal chow; b, vs. sham-operated rats fed fructose-enriched diet.

Fig. 2. Correlations of plasma leptin concentrations with (A) parametrial fat-pad mass or (B) body wt gain in all animals. Sham-operated rats fed normal chow, ○; VMH-lesioned rats fed normal chow, ●; sham-operated rats fed fructose, ◦; VMH-lesioned rats fed fructose, ■; sham-operated rats fed glucose, △; VMH-lesioned rats fed glucose, ▲.
Insulin resistance evaluated by the SSPG method. SSPG values, an indicator of insulin resistance, are depicted in Fig. 3. The values were not increased in VMH-lesioned rats fed normal chow 2 wk after creation of the lesions compared with sham-operated controls. Fructose feeding significantly elevated SSPG values in sham-operated and VMH-lesioned rats compared with those in the chow-fed counterparts. In contrast, glucose feeding significantly decreased SSPG values in both sham-operated and VMH-lesioned rats compared with those in chow-fed counterparts. There was no significant correlation between SSPG values and plasma leptin concentrations (Fig. 3).

Plasma insulin levels and insulin and glucose responses in IVGTT. Plasma insulin levels are shown in Table 1. These levels doubled in VMH-lesioned rats compared with sham-operated controls. Fructose feeding substantially elevated insulin levels in both lean and VMH-lesioned obese rats, whereas glucose feeding did not significantly affect the insulin levels of either of these groups. There was a significant correlation between plasma leptin and insulin levels in all animals. (n = 92, r = 0.500, P < 0.0001), and this correlation became closer when insulin-resistant fructose-fed rats were excluded (n = 72, r = 0.547, P < 0.0001) (data not shown).

Plasma glucose and insulin levels before and after intravenous glucose administration are illustrated in Fig. 4. The plasma glucose response was similar in all groups of rats, except that it was significantly increased in VMH-lesioned rats fed a fructose-enriched diet. The magnitude of the insulin response in IVGTT was significantly increased by fructose feeding: VMH-lesioned rats had twofold greater insulin area under the curve (AUCins) than sham-operated controls. Fructose feeding markedly elevated the insulin area in lean and VMH-lesioned rats, whereas glucose feeding did not alter AUCins in these animals.

Pair-feeding study. In some VMH-lesioned rats (n = 7), food intake (15 g/24 h, normal rat chow) was given to the same number of sham-operated control rats for 2 wk. During the food restriction, their body weight gain became similar to controls (1.5 ± 0.2 vs. 1.3 ± 0.2 g/day), and the parametrial fat-pad weight at 2 wk after the operation was comparable to that of sham-operated controls (2.8 ± 0.3 vs. 3.0 ± 0.4 g). Despite the lack of increased adiposity, these VMH-lesioned rats still had threefold higher levels of plasma leptin than sham-

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**Fig. 3.** Steady-state plasma glucose (SSPG) method for evaluating insulin resistance (top), and correlation between SSPG and plasma leptin concentration (bottom). Rats were given constant infusion of glucose (8 mg·kg⁻¹·min⁻¹), insulin (2.5 mU·kg⁻¹·min⁻¹), and somatostatin (0.5 µg/min) for 170 min, and average concentration of plasma glucose at 150, 160, and 170 min was used to determine SSPG values. Data represent means ± SE; nos. in bars, nos. of rats/group. Statistical significance by one-way ANOVA at P < 0.05: a, vs. sham-operated rats fed normal chow; b, vs. VMH-lesioned rats fed normal chow; c, vs. sham-operated rats fed fructose-enriched diet; d, vs. VMH-lesioned rats fed fructose-enriched diet. Sham-operated rats fed normal chow, ○; VMH-lesioned rats fed normal chow, ●; sham-operated rats fed fructose, ■; VMH-lesioned rats fed fructose, □; sham-operated rats fed glucose, ▲; VMH-lesioned rats fed glucose, △. NS, not significant.
operated controls (10.3 ± 1.9 vs. 2.6 ± 0.3 ng/ml, P < 0.005). These animals had higher plasma insulin levels (2.0 ± 0.2 vs. 1.1 ± 0.8 ng/ml, P < 0.05) and lower SSPG levels (7.9 ± 1.0 vs. 12.1 ± 2.3 mM, n = 2) compared with those in sham-operated controls.

DISCUSSION

It has been reported that sugar-enriched diets cause enlargement of various adipose tissues in rats (13). In addition, Mueller et al. (19) have reported that addition of glucose and fructose to cultured medium stimulates leptin secretion by isolated rat adipocytes. Therefore, we expected that both high glucose and fructose feeding would increase plasma leptin levels in rats in vivo. Contrary to our speculation, however, fructose feeding did not, however, further increase fat-pad weight or plasma leptin levels in VMH-lesioned animals. This is probably because leptin production is fully stimulated by substantial adiposity in these obese animals, so that the effect of dietary glucose on adipose tissue might be masked.

A number of previous studies have demonstrated that plasma insulin concentration rises substantially after the creation of VMH lesions in rats (7, 22), and the subsequent chronic hyperinsulinemia is thought to be an essential factor in the development of obesity in the lesioned rats (10). A number of recent studies have shown that insulin markedly increases leptin production in cultured adipocytes (25) (1) and that persistent infusion of insulin increases plasma leptin levels in vivo (15, 17, 21). VMH-lesioned rats with pair feeding still had higher leptin and insulin levels, although their body weight gain and parametrial fat-pad mass were comparable to those in sham-operated controls. Therefore, the hyperinsulinemia developed in VMH-lesioned rats may be directly associated with hyperleptinemia apart from their increased adiposity. Because the fructose moiety per se does not stimulate insulin secretion by pancreatic islets, the hyperinsulinemia in fructose-fed rats must result from the developed insulin resistance (28). In fact, the SSPG value, an indicator of insulin resistance, was elevated substantially by fructose feeding in the present study. Fasting insulin levels or insulin responses in IVGTT also rose substantially in association with increased insulin resistance. Both sham-operated and VMH-lesioned rats fed fructose had
higher insulin levels at 30 and 60 min but not at the usual peak time of 5 min. The reason that the time of maximal insulin secretion was delayed in the fructose-fed animals remains unknown. A possible explanation is that fructose feeding might decrease the efficacy of insulin extraction by the liver, which retards insulin clearance from the circulation. It should be noted that the substantial hyperinsulinemia caused by fructose feeding did not lead to a rise in plasma leptin levels in either lean or obese rats. This suggests that hyperinsulinemia compensating for insulin resistance cannot stimulate leptin production by adipocytes; in other words, insulin resistance attenuates insulin-induced leptin production. Mueller et al. (19) reported recently that leptin production in cultured adipocytes is more closely associated with intracellular glucose metabolism than with insulin concentration in the cultured medium. Insulin resistance impedes glucose uptake and its utilization in adipocytes; therefore, it is reasonable to assume that leptin production is decreased in insulin-resistant adipocytes such as those of fructose-fed rats (16). Several clinical studies have suggested that insulin resistance is associated with hyperleptinemia (8, 23); however, adiposity and insulin resistance are usually associated with, and both correlate with, leptin levels, making it difficult to interpret the relation between hyperinsulinemia and hyperleptinemia. Unlike in congenitally obese animals, insulin resistance was not developed in VMH-lesioned rats during the dynamic phase of obesity. Therefore, the VMH-lesioned rat would be a useful animal with which to explore the direct relationship between plasma leptin and adiposity excluding insulin resistance. Although insulin resistance was not developed in VMH-lesioned rats, plasma leptin levels were elevated substantially with increased adiposity. This clearly indicates that insulin resistance is not always associated with hyperleptinemia. In the present study, insulin sensitivity in VMH-lesioned rats was impaired by fructose feeding but was, in turn, stimulated by glucose feeding, whereas plasma leptin levels were not altered by either sugar-enriched diet. There was no correlation between the SSPG values and plasma leptin levels in all animals. These results provide additional evidence that insulin resistance plays only a small role in regulating leptin production.

The VMH is a key target site of leptin; thus the creation of VMH lesions cause rats to become completely leptin resistant. Because VMH-lesioned rats lack the normal homeostasis for leptin production (mainly adipose tissues) between the central nervous system and the peripheral organs, their plasma leptin levels would be upregulated by the destruction of the key target organ of this hormone. Therefore, the present results may not be readily applicable to other obese animals in which the homeostasis of leptin is preserved. To explore the effect of dietary sugars on plasma leptin levels in obese animals, more studies will be required with congenitally or dietarily obese animals. Nevertheless, the present study may provide useful information about the direct effect of dietary sugars on leptin production by adipose tissues.

In summary, in lean rats, dietary glucose increased plasma leptin levels, whereas dietary fructose did not alter the levels, although it did produce significant hyperinsulinemia and insulin resistance. In VMH-lesioned obese rats, neither glucose- nor fructose-enriched diet affected plasma leptin levels significantly. Thus dietary sugars modify leptin production in lean animals, but they do not affect it in VMH-lesioned obese animals, in which leptin production is remarkably stimulated, because of the lack of normal leptin feedback. The results also suggest that insulin resistance and hyperinsulinemia compensated for insulin resistance do not stimulate leptin production.

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