Hepatic steatosis and plasma dyslipidemia induced by a high-sucrose diet are corrected by an acute leptin infusion

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Huang W, Dedousis N, O’Doherty RM. Hepatic steatosis and plasma dyslipidemia induced by a high-sucrose diet are corrected by an acute leptin infusion. J Appl Physiol 102: 2260–2265, 2007. First published March 15, 2007; doi:10.1152/japplphysiol.01449.2006.—High sucrose (HS) feeding in rats induces hepatic steatosis and plasma dyslipidemia. In previous reports (Huang W, Dedousis N, Bhatt BA, O’Doherty RM. J Biol Chem 279: 21695–21700, 2004; and Huang W, Dedousis N, Bandi A, Lopaschuk GD, O’Doherty RM. Endocrinology 147: 1480–1487, 2006), our laboratory demonstrated a rapid (~100 min) leptin-induced decrease in liver and plasma VLDL triglycerides (TG) in lean rats, effects that were abolished in obese rats fed a high-fat diet, a model that also presents with hepatic steatosis and plasma dyslipidemia. To further examine the capacity of acute leptin treatment to improve metabolic abnormalities induced by nutrient excess, hepatic leptin action was studied in rats after 5 wk of HS feeding. HS feeding induced hepatic steatosis (TG +80 ± 8%; P = 0.001), plasma hyperlipidemia (VLDL-TG +102 ± 14%; P = 0.001), hyperinsulinemia (plasma insulin +67 ± 12%; P = 0.04), and insulin resistance as measured by homeostasis model assessment (+125 ± 20%; P = 0.02), without increases in adiposity or plasma leptin concentration compared with standard chow-fed controls. A 120-min infusion of leptin (plasma leptin 13.6 ± 0.7 ng/ml) corrected hepatic steatosis (liver TG −29 ± 3%; P = 0.003) and plasma hyperlipidemia in HS (VLDL-TG −42 ± 4%; P = 0.001) and increased plasma ketones (+45 ± 3%; P = 0.006), without altering plasma glucose, insulin, or homeostasis model assessment compared with saline-infused HS controls. In addition, leptin activated liver phosphatidyl-inositol 3-kinase (P = 0.01) and protein kinase B (Akt; +90 ± 29%; P = 0.02), and inhibited acetyl-CoA carboxylase (40 ± 7%; P = 0.04) in HS, further demonstrating that hepatic leptin action was intact in these animals. We conclude that 1) leptin action on hepatic lipid metabolism remains intact in HS-fed rats, 2) leptin rapidly reverses hepatic steatosis and plasma dyslipidemia induced by sucrose, and 3) the preservation of hepatic leptin action after a HS diet is associated with the maintenance of low adiposity and plasma leptin concentrations.

Previous studies from our group (17, 18) demonstrated a rapid (90–120 min) leptin-induced depletion of hepatic triglycerides (TG) and a lowering of plasma VLDL-associated TG (VLDL-TG) in lean rats. These effects resulted from direct action of leptin at the liver and required the activation of liver phosphatidylinositol 3-kinase (PI3-kinase), inactivation of acetyl-CoA carboxylase (ACC) and subsequent stimulation of liver fatty acid oxidation and ketogenesis (17, 18). Interestingly, the metabolic and biochemical effects of leptin at the liver were abrogated in rats fed a high-fat (HF) diet for 5 wk or a “cafeteria” diet for as few as 3 days (17, 18, 36), resulting in the maintenance of hepatic steatosis and plasma hypertriglyceridemia in the HF-fed animals. Together, these studies suggest that exposure to energy excess/nutrient oversupply rapidly induces leptin resistance. However, it is unclear whether these observations can be generalized to other nutrient regimes that induce dyslipidemia. In this respect, fructose, as a component of corn syrup or sucrose, is a major ingredient of many processed foods and has been proposed to contribute to the development of obesity and dyslipidemia in humans (5, 6, 10, 12, 14). Indeed, diets rich in this sugar induce hepatic steatosis and plasma hyperlipidemia in rodents (2, 20, 25, 31). However, the effects of fructose feeding on hepatic leptin action or the capacity of leptin to ameliorate the metabolic dysregulation induced by fructose have not been investigated. The present study was undertaken to address these issues. Specifically, hepatic leptin action was assessed in vivo in rats after 5 wk of a high-sucrose (HS) diet. The data demonstrate that 1) leptin action on hepatic lipid metabolism remains intact in HS-fed rats, 2) leptin rapidly reverses hepatic steatosis and plasma dyslipidemia induced by sucrose, and 3) the preservation of hepatic leptin action after a HS diet is associated with the maintenance of basal adiposity and plasma leptin concentrations in this model.

MATERIALS AND METHODS

Animal care and maintenance. Male Wister rats were purchased from Charles River (Madison, WI) at a weight of 175–200 g. After arrival, rats were maintained on a constant 12:12-h light-dark cycle with free access to water and were ad libitum fed with a standard chow (SC) diet (11% of calories from fat), or a HF diet (Harlan Teklad, Madison WI; TD 96001, 45% of calories from fat), or a HS diet (Research Diet; 68% of calories from sucrose and 11% of calories from fat) for 5 wk. All procedures were approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh, and they were in accordance with the National Research Council’s Guide for the Care and Use of Laboratory Animals.

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Implantation of chronic indwelling catheters. Animals were anesthetized with a ketamine-xylazine-acepromazine mix (60 mg/kg ketamine, 5 mg/kg xylazine, 1 mg/kg acepromazine). Catheters (PE-50, Intramedic, Becton Dickinson, Sparks, MD) were introduced into the left carotid artery (advanced to the aortic arch) and the right jugular vein (advanced to the right atrium) as previously described (7, 17). The catheters were exteriorized at the back of the neck, filled with a sterile 3:1 glycerol-heparin mix, and flame sealed. The wounds were closed with sutures and treated with Betadine. The animals were treated with ketoprofen (2 mg/kg sq), and their recovery was monitored with special attention given to food intake, weight gain, and healing of wounds. Animals were allowed at least 4 days to recover and only those that had achieved >90% of presurgery weights were used in the studies.

Experimental design. Conscious 18-h-fastfed HS-fed or SC-fed rats received a continuous intravenous infusion of either vehicle (saline) or leptin using a syringe pump (model 11, Harvard Apparatus, Dover, MA) at a rate of 0.2 µg·kg⁻¹·min⁻¹ for 120 min (preceded by a 2-min priming dose of 2 µg·kg⁻¹·min⁻¹) or for 15 min in the experiments designed for measuring liver PI3-kinase activity and protein kinase B (Akt) phosphorylation (17, 18). Volume delivery was 5 ml·kg⁻¹·h⁻¹ for both vehicle and leptin infusions. Blood samples were taken before infusions and at 120 min; the rats were then anesthetized; and the liver and skeletal muscle (soleus) were promptly removed, snap frozen in liquid nitrogen, and stored at −80°C until analysis.

Tissue and plasma measurements. Tissue triglycerides were determined as described previously (7, 17). Briefly, ~50 mg of frozen liver or muscle tissue were extracted in 1 ml of a chloroform-methanol mix (2:1). After redissolving of the lipid pellet in 60 µl of tert-butanol and 40 µl of a Triton X-114-methanol (2:1) mix, the triglycerides were measured spectrophotometrically (DU 530, Beckman Coulter, Fullerton, CA) using the glycerol phosphate oxidase-triglyceride kit and Lintrol lipids as standard (Sigma-Aldrich).

Apolipoprotein B-containing lipoproteins in plasma (200 µl, 1:3 diluted with saline) were precipitated as described earlier (17, 37). Briefly, 5% by volume of 2 g/dl dextran sulfate and 10% by volume of 2.25 M magnesium sulfate were added to the sample, and after gentle mixing, a 10-min incubation at room temperature, and centrifugation at 2,000 g for 10 min, the supernatant was removed and the pellet was dissolved in 200 µl of a high-salt buffer (containing 2 M NaCl, 2 mM EDTA, and 50 mM NaPi), 200 µl tert-butanol and 100 µl of a 1:1 methanol-Triton X-100 mix, each added separately followed by a 30-s vortex. After centrifugation at 4,000 g, triglyceride in the upper phase of the supernatant was analyzed spectrophotometrically as described above.

Plasma β-hydroxybutyrate was measured enzymatically using the β-hydroxybutyrate liquidcolor kit (Stanbio, Boerne, TX), plasma glucose concentration was determined using a glucose analyzer (Glucose 2, Beckman Coulter). Leptin and insulin were measured using rat-specificRIA kits (Linco Research, St. Charles, MO). Homeostasis model assessment (HOMA) was calculated as follows:

\[
\text{HOMA} = \frac{\text{Insulin} \cdot \text{Plasma Glucose}}{22.5}
\]

Table 1. Basal metabolic variables in rats fed a standard chow, high-sucrose, or high-fat diet for 5 wk

<table>
<thead>
<tr>
<th></th>
<th>Body Weight, g</th>
<th>Epididymal Fat, g</th>
<th>Leptin, ng/ml</th>
<th>Insulin, µU/ml</th>
<th>Plasma Glucose, mmol/l</th>
<th>HOMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC</td>
<td>369 ± 11</td>
<td>5.7 ± 0.3</td>
<td>1.4 ± 0.3</td>
<td>16.8 ± 3</td>
<td>5.4 ± 0.2</td>
<td>3.4 ± 0.6</td>
</tr>
<tr>
<td>HS</td>
<td>377 ± 8</td>
<td>6.0 ± 0.7</td>
<td>1.8 ± 0.9</td>
<td>27.8 ± 3*</td>
<td>5.7 ± 0.1</td>
<td>7.3 ± 1*</td>
</tr>
<tr>
<td>HS + saline</td>
<td>NA</td>
<td>NA</td>
<td>1.5 ± 0.2</td>
<td>30.8 ± 5.9*</td>
<td>6.1 ± 0.3</td>
<td>8.6 ± 2.1*</td>
</tr>
<tr>
<td>HS + leptin</td>
<td>NA</td>
<td>NA</td>
<td>13.6 ± 0.7*</td>
<td>26.5 ± 3.4*</td>
<td>5.6 ± 0.1</td>
<td>5.9 ± 0.5*</td>
</tr>
<tr>
<td>HF</td>
<td>456 ± 49†</td>
<td>18.7 ± 1.1†</td>
<td>6.4 ± 1.2†</td>
<td>48.0 ± 10.2*</td>
<td>7.1 ± 3.0†</td>
<td>15.1 ± 0.2†</td>
</tr>
</tbody>
</table>

Table 1. Basal metabolic variables in rats fed a standard chow, high-sucrose, or high-fat diet for 5 wk

**Values are means ± SE; n = minimum 6/group. All data are from fasted (18 h) rats. SC, standard chow; HS, high sucrose; HF, high fat; NA, not applicable.**

**Leptin corrects hepatic steatosis and plasma hypertriglyceridemia in HS-fed animals.** To address the effects of sucrose on hepatic leptin action, leptin was infused into overnight-fastfed rats. Hepatic steatosis and plasma triglyceride concentrations were similarly decreased in HS- and SC-fed animals (Table 1). Next we compared the effects of high sucrose feeding with the effects of 5 wk of HF feeding on these metabolic variables, because our laboratory has previously reported (17, 18) that hepatic leptin action is completely absent in HF-fed animals. The dyslipidemia effects of HF were similar to HS (Table 1, Fig. 1). However, there was a trend for plasma insulin to be increased (+89 ± 38%; P = 0.07) and significant elevations in plasma glucose (+24 ± 3%; P = 0.002), resulting in an elevated HOMA (+129 ± 46%; P = 0.004), on the HF diet compared with a HS diet. Furthermore, HF feeding induced substantial increases in body weight (+23 ± 2%; P = 0.0001), epididymal fat pad weight (+214 ± 4%; P = 0.0001), and fasting leptin concentrations (+215 ± 69%; P = 0.01) compared with HS feeding (Table 1).
fasted HS-fed animals at a rate that increased the plasma leptin concentration to 13.6 ± 0.7 ng/ml, comparable to that achieved in previous studies and within a physiological range (17, 18). Leptin decreased liver TG levels in HS-fed animals by 29 ± 3% (P = 0.003) and plasma VLDL-TG concentration by 42 ± 4% (P = 0.001), similar to decreases observed in SC-fed rats (Fig. 2, A and C). Indeed, the decreases induced by leptin were sufficient to return TG levels to those observed in SC-fed animals. These effects are in stark contrast to the loss of leptin action in HF-fed rats. Unlike the effects of leptin on TG in liver and plasma, acute leptin exposure did not decrease skeletal muscle TG levels in HS or SC-fed animals (Fig. 2B), in agreement with our laboratory’s previous observations (17), nor were insulin, glucose, or HOMA altered by leptin in HS-fed animals (Table 1).

**DISCUSSION**

The present study is an extension of previous studies (17, 18) that addressed the acute effects of leptin on hepatic lipid metabolism in lean and obese rats, and the capacity of leptin to correct the dyslipidemia induced by nutrient excess, in this case a HS diet. A number of novel observations are presented, namely that 1) leptin action on hepatic lipid metabolism remains intact in HS-fed rats; 2) leptin rapidly reverses the hepatic steatosis and plasma dyslipidemia induced by sucrose, and 3) the preservation of hepatic leptin action in HS-fed animals is associated with the maintenance of basal adiposity and plasma leptin concentrations.

The effects of leptin in SC-fed rats on liver lipid metabolism requires the activation of PI3-kinase, inactivation of ACC, and stimulation of oxidative pathways (17, 18). In HF-fed obese animals, the effects of leptin are abolished, most likely result-
The simplest explanation is that increases in leptin levels are a driving force for the development of leptin resistance in HF feeding. Thus, if leptin levels do not increase, leptin sensitivity will be maintained.

The maintenance of hepatic leptin action in HS-fed animals enables the correction of the liver and plasma hyperlipidemia by leptin in these animals. The rapidity with which this dysmetabolic phenotype is corrected is striking, requiring only a 120-min leptin exposure. It is worthy of note that these observations may have implications for human disease states where steatosis and dyslipidemia occur in the absence of obesity. Interest in a therapeutic role for leptin has recently been rekindled due to its capacity to correct metabolic abnormalities in lipodystrophy, where there are low circulating leptin concentrations (19, 24, 27, 29) and in Rabson-Mendenhall syndrome (severe insulin resistance with insulin-receptor mutation) (9, 38). Clearly, leptin may have beneficial metabolic effects in other situations of dyslipidemia, with the caveat that leptin resistance should be absent, i.e., in states other than obesity, a leptin-resistant situation.

It has been proposed that leptin resistance/leptin absence contributes to the hepatic steatosis observed in obesity and lipodystrophy. A corollary of this hypothesis is the often-debated concept of lipotoxicity, whereby it is proposed that a
The primary function of leptin is to prevent an inappropriate accumulation of lipid in nonadipose tissues, with skeletal muscle, liver, and β-cells being the tissues most often cited as examples of this phenomenon (32–34). Supporting this hypothesis is the well-described “lipogenic” effects of leptin, the presence of substantial dyslipidemia when leptin action is impaired (leptin resistance) or absent, and the dramatic improvements in lipid profiles when leptin is administered in cases of lipodystrophy or genetic absence of leptin (3, 11, 24, 27–29). Data obtained in the present study do not comfortably fit into this simple scheme. In support of the hypothesis, the capacity of leptin to reverse steatosis and plasma dyslipidemia induced by HS feeding is demonstrated, because acute leptin administration returns liver and circulating lipid levels to near control values in HS-fed animals. However, it is also clear that hepatic steatosis is occurring in the HS-fed animal despite the maintenance of leptin sensitivity. Three explanations can be proposed to explain these observations. First, the HS-diet has a very high lipogenic capacity, arising predominantly from the dramatic increase in glycolytic flux caused by the bypassing by fructose of a rate-determining enzymatic reaction (phosphofructokinase) and fructose-6-phosphate-stimulated recruitment of glucokinase to the cytoplasm (4, 16, 22). Much of this flux is directed into lipogenesis in the rat, presumably because of a supply of glycolytic intermediates that are in excess of what can be metabolized by the liver through nonlipogenic pathways. Second, there may be an inadequate secretion of leptin in response to elevated fructose intake. In support of this possibility is the observation in humans that the postprandial leptin increase was less in response to fructose intake compared with glucose intake (30). Finally, the hyperinsulinemia present in HS-fed animals may drive an increase in lipogenesis that counters the antilipogenic effects of leptin.

At first glance, the lack of effects of acute leptin treatment on the elevated skeletal muscle TG and plasma insulin concentration in the HS diet are surprising, given the well-described effects of leptin treatment on these variables in rodents. In the case of muscle TG, it is important here to make a distinction between acute and chronic effects of leptin. Chronic leptin administration lowers muscle TG (7). However, acute leptin administration has no effect on muscle TG (17), despite stimulating fatty acid oxidation (23). These data suggest that the acute lipid-lowering effects of leptin are particular to aspects of liver lipid metabolism that are not shared with skeletal muscle, such as lipoprotein metabolism. In the case of insulin, it has been reported that leptin acutely decreases the plasma insulin concentration during a hyperglycemic clamp (8). However, the acute effects of leptin on plasma insulin concentration under basal (fasted) conditions or in insulin-resistant states have not been reported. The present study suggests that longer term administration of leptin is required to illicit improvements in fasting hyperinsulinemia.

In conclusion, the present study demonstrates that hepatic steatosis and plasma dyslipidemia induced by sucrose feeding is rapidly reversible by leptin. These effects were observed in the absence of alterations in body weight, epididymal fat pad weight, and plasma leptin concentration, suggesting that in situations of dyslipidemia and normal leptin sensitivity, leptin is an extremely effective intervention for acutely lowering plasma and liver lipid levels.


