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

Wan Huang, Nikolas Dedousis, and Robert M. O’Doherty

1Division of Endocrinology, Department of Medicine, and 2Department of Molecular Genetics and Biochemistry, University of Pittsburgh, Pittsburgh, Pennsylvania

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 concentrations. 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 phosphatidylinositol 3-kinase (70 ± 18%; 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 Wistar 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.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
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, MA) 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-fasted 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 determined as previously described (17, 18). Phospho-Akt (Ser-473), total Akt, and phospho-ACC (Ser-79) were measured using standard immunoblotting techniques as previously described [α-phospho-Akt catalog no. 9271, α-Akt, catalog no. 9272 (Cell Signaling, Beverly, MA); α-phospho-ACC catalog no. 07-303 (Upstate Biotechnology, Lake Placid, NY), all used at 1:1,000 dilution].

Statistical methods. All results are expressed as means ± SE. Statistical significance was determined by t-test using the Systat statistical program (Evanston, IL). Statistical significance was assumed at P < 0.05.

RESULTS

Five weeks of HS feeding induces plasma hyperlipidemia, hepatic steatosis, and insulin resistance without altering body weight, adiposity, or plasma leptin concentration. We first established the effects of HS feeding on measures of lipid and glucose metabolism and body composition. As previously described in rodents (2, 20, 25, 31), 5 wk of a HS diet induced hypertriglyceridemia (plasma VLDL-TG +102 ± 14%, P = 0.001), hepatic and skeletal muscle steatosis (liver TG +80 ± 8%; P = 0.001, skeletal muscle TG +162% ± 45%; P = 0.03), hyperinsulinemia without attendant increases in plasma glucose (fasting plasma insulin +67 ± 12%; P = 0.04), and increased HOMA, a measure of insulin resistance (+125 ± 20%; P = 0.02), compared with SC-fed controls (Table 1, Fig. 1). Body weight, epididymal fat pad weight, and fasting leptin concentrations were similar in HS- and SC-fed animals (Table 1). We next 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 ( 4%/ P < 0.01) and fasting leptin concentrations ( 4% ; P = 0.01), compared with HS feeding (Table 1).

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-fasted rats (18 h) on SC, standard chow; HS, high sucrose; HF, high fat; NA, not applicable. HS + saline and HS + leptin groups received a continuous intravenous delivery of either vehicle (saline) or leptin (leptin) at the rate of 0.2 μg·kg⁻¹·min⁻¹ following a priming dose of 2 μg·kg⁻¹·min⁻¹ in a volume of 5 ml·kg⁻¹·h⁻¹, for 120 min. †Significant difference between the HF and HS groups, P < 0.05. *Significant difference between HS or HF and SC groups, P < 0.05. Homeostasis model assessment (HOMA) was calculated as follows: plasma glucose concentration (in mmol/l) was multiplied by the fasting plasma insulin concentration (in μU/ml) and divided by the constant 22.5 as previously described (21).

Insulin receptor substrate-1-associated PI3-kinase activity was determined as previously described (17, 18). Phospho-Akt (Ser-473), total Akt, and phospho-ACC (Ser-79) were measured using standard immunoblotting techniques as previously described [α-phospho-Akt catalog no. 9271, α-Akt, catalog no. 9272 (Cell Signaling, Beverly, MA); α-phospho-ACC catalog no. 07-303 (Upstate Biotechnology, Lake Placid, NY), all used at 1:1,000 dilution].

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 ± 1.1*</td>
</tr>
<tr>
<td>HS + leptin</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>
<td>15.1 ± 0.2*</td>
</tr>
<tr>
<td>HF</td>
<td>456 ± 9*†</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>

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. HS + saline and HS + leptin groups received a continuous intravenous delivery of either vehicle (saline) or leptin (leptin) at the rate of 0.2 μg·kg⁻¹·min⁻¹ following a priming dose of 2 μg·kg⁻¹·min⁻¹ in a volume of 5 ml·kg⁻¹·h⁻¹, for 120 min. †Significant difference between the HF and HS groups, P < 0.05. *Significant difference between HS or HF and SC groups, P < 0.05. Homeostasis model assessment (HOMA) was calculated as follows: plasma glucose concentration (in mmol/l) was multiplied by the fasting plasma insulin concentration (in μU/ml) and then divided by a constant 22.5 (21).
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% (P < 0.003) and plasma VLDL-TG concentration by 42% (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 (17, 18) 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).

Leptin activates liver PI3-kinase and Akt and increases oxidative metabolism in high-sucrose-fed rats. Our laboratory has identified (17, 18) increased fatty acid oxidation and ketogenesis mediated by activation of the PI3-kinase-Akt-ACC axis as a mechanism for the acute lipid-lowering effect of leptin in liver. Consistent with these studies, leptin increased liver PI3-kinase activity (70 ± 18%; P = 0.01) and phosphorylation of Akt (90 ± 29%; P = 0.02) in HS-fed rats compared with the saline-infused rats fed the same diet (Fig. 3). Furthermore, leptin treatment increased liver ACC carboxylase phosphorylation (40 ± 7%; P = 0.04) and plasma ketone concentrations (45 ± 3%; P = 0.006) compared with saline controls (Fig. 4). Together, these data confirm that the biochemical mechanisms of leptin action in liver remain intact in the sucrose fed rat and are likely to mediate the reversal of hepatic steatosis and plasma hyperlipidemia in these animals.

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-
ing from the actions of an unknown inhibitory factor on leptin activation of PI3-kinase (17, 18). It is apparent in the present study that the capacities of leptin to activate PI3-kinase, inactivate ACC and stimulate oxidative pathways remain intact in HS-fed animals. This is the case despite the capacity of sucrose to induce a similar extent of dyslipidemia to that observed with HF feeding. However, it is also apparent that there are substantial differences in phenotype induced by a 5-wk HF diet compared with a HS diet of similar duration that may explain the maintenance of leptin sensitivity in HS-fed animals. Of particular note are the differences in adiposity and plasma leptin concentrations between these two models. Thus there are approximately threefold increases in visceral adiposity and leptin concentrations with HF feeding, whereas there are little or no increases in these variables in HS-fed rats. The fact that leptin concentrations do not increase is indicative that leptin sensitivity is maintained in these animals, as our data demonstrate. However, it is unclear why leptin sensitivity should be altered by HF feeding but not by HS feeding. Perhaps 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

![Fig. 3. Effects of leptin on liver phosphatidylinositol 3-kinase (PI3K) activity and protein kinase B (Akt) phosphorylation in HS-fed rats. Fasted (18 h) HS-fed rats received a continuous intravenous infusion of either vehicle (Leptin −) or leptin (Leptin +) for 15 min as described in the legend of Fig. 2. Subsequently, liver PI3K activity (A) and Akt phosphorylation (B) were measured as described in MATERIALS AND METHODS. A (top): representative autoradiograph of PI3K activity. B (bottom): quantification of PI3K activity measured as phosphatidylinositol trisphosphate [P(3)P] production and derived from scintillation counting of 32P-containing P(3)P on thin-layer chromatography plates. B (top): representative autoradiograph of phosphorylated-Akt (phospho-Akt; P-Akt; Ser473) and total Akt. B (bottom): quantification of phosphorylated-Akt normalized to total AKT levels as measured by densitometry. Values are means ± SE; n = a minimum of 5/group for all measurements. IP, immunoprecipitated; α-IRS-1, α-insulin receptor substrate-1. Significant differences are indicated.](http://jap.physiology.org/)
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 “lipopenic” 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.

ACKNOWLEDGMENTS

We thank Darinka Sipula and John Dube for technical support.

GRANTS

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) Grant R01 DK-072162 (to R. M. O’Doherty) and by a University of Pittsburgh Obesity and Nutrition Research Center (NIDDK Grant P30 DK-462) Pilot and Feasibility award (to W. Huang). W. Huang was also supported by NIDDK Grant T32-DK-07052 (Research Training in Diabetes and Endocrinology).

REFERENCES


