Leptin has acute effects on glucose and lipid metabolism in both lean and gold thioglucose-obese mice

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Bryson, Janet M., Jenny L. Phuyal, Verity Swan, and Ian D. Caterson. Leptin has acute effects on glucose and lipid metabolism in both lean and gold thioglucose-obese mice. Am. J. Physiol. 277 (Endocrinol. Metab. 40): E417–E422, 1999.—Leptin is reported to have effects in peripheral tissues that are independent of its central effects on food intake and body weight. In this study, the acute effects of a single dose of recombinant mouse leptin on lipid and glucose metabolism in lean and gold thioglucose-injected obese mice were examined. Changes were measured 2 h after leptin injection. In lean mice, liver and white adipose tissue (WAT) lipogenesis was inhibited. The activity of the pyruvate dehydrogenase complex (PDHCa), the rate-determining step for glucose oxidation, was reduced in heart, liver, quadriceps muscle, and both brown and white adipose tissues. Muscle and liver glycogen and liver triglyceride (TG) content was unchanged, but muscle TG was decreased. In obese mice, liver and WAT lipogenesis was inhibited and PDHCa reduced in heart and quadriceps muscle. Muscle and liver glycogen was decreased but not TG. Serum insulin was reduced in obese but not lean mice. These results are consistent with a role for leptin in the maintenance of steady-state energy stores by decreasing lipid synthesis and increasing fat mobilization, with decreased glucose oxidation occurring as a result of increased fatty acid oxidation.

lipogenesis; pyruvate dehydrogenase complex; fatty acid oxidation

The effects of leptin on food intake, energy expenditure, and body weight are mediated by leptin receptors situated in the hypothalamus, with a more potent anorexic effect being achieved when leptin is administered centrally rather than peripherally (38). Leptin has also been shown to have effects on whole body glucose and lipid metabolism that are independent of its effects on food intake (21) and that may occur before any loss of body weight (16). The presence of OB receptors in peripheral tissues (10, 20, 39) suggests that the effects of leptin on metabolic events may be due to direct actions of leptin in individual tissues. However, results of in vitro studies of leptin action are contradictory. In skeletal muscle, leptin has been reported to alter lipid partitioning (26); to stimulate (1, 14), inhibit (41), or have no effect on (13, 29) glucose transport; to stimulate (1), inhibit (22), or have no effect on (13, 26) glycogen synthesis; and to have no effect on glucose oxidation (26). Similarly, in adipocytes, leptin has been shown to have no effect (41) or an inhibitory (25) effect on insulin-stimulated glucose and lipid metabolism, as well as a stimulatory effect on lipolysis (12, 34). In whole body studies, acute leptin infusion has been shown to increase glucose turnover and uptake (16), improve insulin sensitivity during euglycemic hyperinsulinemic clamps (35), or to have no effect on glucose uptake in peripheral tissues while enhancing hepatic insulin action (32).

The gold thioglucose (GTG) mouse is a chemically induced model of obesity in which an infarction of the hypothalamus results in hyperphagia and weight gain (2). This lesion causes a reduction in the number of leptin receptors in the hypothalamus (11). The presence of leptin receptors in other tissues that are not affected by GTG injection makes the GTG-obese mouse a useful model for studying the peripheral effects of leptin independent of any hypothalamic actions. The aims of this study were to determine the effects of acute leptin administration on different parameters of lipid and glucose metabolism in both lean and GTG-injected obese mice. We hypothesized that if leptin is an indicator of body adiposity, then artificial elevation of leptin would result in decreased lipogenesis and increased mobilization of lipid stores. The resulting increase in the availability of fatty acids for oxidation would have an indirect effect on glucose oxidation, with a reduction in the activity of the rate-determining enzyme of glucose oxidation, the pyruvate dehydrogenase complex (PDHC).

METHODS

Animals. Male CBA/T6 mice were obtained at 6 wk of age from the Blackburn Animal House, University of Sydney. Obesity was induced by a single intraperitoneal injection (0.5 g/kg) of GTG (Sigma Chemical, St. Louis, MO). Mice were kept on a 12:12-h light-dark cycle, with the light cycle between 0600 and 1800, and were allowed free access to food and water. Experiments were performed 10 wk after GTG injection, when obesity and insulin resistance are fully developed (2). The total number of mice in each group ranged from 18 to 27.

Acute leptin administration. All experiments were performed between 0900 and 1100, with the mice having free access to food and water until the start of the experiment. A single dose (25 µg/mouse) of recombinant mouse leptin (Amgen, Thousand Oaks, CA) was injected intraperitoneally to one-half of the GTG-injected mice and one-half of the age-matched controls. Mice were killed 2 h after leptin administration. Subsets of the leptin-injected mice (both lean and obese) were used for estimation of PDHC activity (PDHCa) in heart, liver, quadriceps muscle (QM), brown adipose tissue (BAT), and epididymal white adipose tissue (WAT). Tissues were removed immediately after animals were killed and were homogenized as detailed in Mitochondrial preparation. Other mice, used for studying the effects of leptin on lipogenesis,
were given an intraperitoneal injection of tritiated water 1 h after the leptin injection and 1 h before these mice were killed. Liver, WAT, and BAT were collected for determination of lipogenic rate, as detailed in Lipogenesis. Blood was collected from the chest cavity of all mice and serum-frozen for subsequent assay of glucose, insulin, nonesterified fatty acids (NEFA), triglycerides (TG), and leptin. Further liver and muscle samples were freeze-clamped for assay of TG and glycogen content.

Lipogenesis. Pieces of liver, BAT, and WAT, which had been frozen at the time animals were killed, were weighed, immediately saponified in ethanolic KOH, and acidified with H2SO4, and fatty acids were extracted in light petroleum (boiling point 60–80°C). Organic extracts were evaporated to dryness, the 3H content of fatty acids was determined by liquid-scintillation counting, and the rate of lipogenesis was determined as previously described (7). The number in each group used for lipogenesis was between 10 and 15.

Mitochondrial preparation. Mitochondria were prepared from heart, liver, QM, BAT, and WAT after immediate homogenization of tissues in ice-cold mitochondrial preparation buffer, as previously reported (4). This buffer consisted of 0.25 M sucrose, 0.5 mM Tris·HCl, 2 mM EGTA, pH 7.5, with 50 mM NaF and 10 mM sodium dithiothreitol added to inhibit interconversion of the PDHC and maintain the complex in its in vivo state. After the homogenate had been centrifuged at 800 g for 10 min, the supernatant was decanted and kept on ice. The pellet was rehomogenized in the preparation buffer and recentrifuged at 800 g. The combined supernatants were spun at 10,000 g, and the resulting mitochondrial pellet was transferred in a small amount of buffer to a microfuge tube, spun at 10,000 g, and stored in liquid nitrogen.

Enzyme assays. Mitochondria prepared from the heart and liver were extracted for enzyme assay by alternate thawing and refreezing in extraction buffer (50 mM potassium phosphate, 10 mM EGTA, and 2 mM dithiothreitol, pH 7.0). PDHCa was assayed spectrophotometrically by coupling the production of acetyl-CoA to the acetylation of the dye p-(p-aminophenylazo)-benzenesulfonic acid with the enzyme aminopyridine transferase (3). Citrate synthase (CS) activity was also measured, and PDHCa was expressed per unit of CS activity to correct for any differences in the recovery or purity of the mitochondrial extracts. The CS activity per milligram protein of the mitochondrial extracts was unchanged by lepin treatment in all tissues tested (data not shown). The number of mice in each group was between 8 and 12.

Tissue analyses. Muscle and liver glycogen content was measured using the filter paper method of Chan and Exton (6). Glycogen content is calculated as moles of glucosyl residues per gram wet weight of tissue. Tissue TG were measured by a colorimetric enzymatic procedure (as for serum analyses) after extraction with chloroform-methanol.

Serum analyses. Serum leptin was measured by RIA (Linco Research, St. Louis, MO). Serum glucose was measured by a glucose oxidase/peroxidase method with 4-aminopyridine as the dye, serum insulin by a double-antibody RIA using rat insulin standards, and anti-rat insulin first antibody (Linco Research) and serum NEFA and TG by commercial in vitro enzymatic colorimetric methods (NEFA: Wako Chemical, Osaka, Japan; TG: Boehringer Mannheim, Mannheim, Germany).

Statistics. All results are given as means ± SE. Comparisons between control (CON) and GTG mice and between treated and untreated groups were performed using Student's t-test or one-way ANOVA by use of the Statview IV statistical package.

RESULTS

Preliminary experiments had shown that serum leptin levels peaked 60 min postinjection at 125–150 ng/ml with no difference between the peak values for lean and obese mice (unpublished data). The serum leptin level remained significantly higher than levels in noninjected lean and obese mice for 4 h. Other preliminary experiments showed that the changes in PDHCa were maximal 2 h postinjection (unpublished data), and so all studies were conducted at that time.

Effect of acute leptin administration on individual tissue weights. The body weight and BAT and WAT weights of lean and obese mice were similar in the untreated and treated groups (Table 1). The liver weight in the treated lean mice was slightly less than in the untreated group.

Effect of acute leptin administration on serum leptin, insulin, and metabolite levels. Serum leptin was significantly higher in the leptin-treated groups (Table 2) and was the same for both lean and obese mice. In the untreated mice, the obese group had leptin levels that were threefold greater than those of the lean mice, as well as significantly higher insulin, glucose, NEFA, and TG than lean mice. Serum insulin levels in lean mice were unaffected by leptin treatment, but there was a significant reduction in insulin levels in the obese mice although they remained hyperinsulinemic. Serum glucose was unchanged in both lean and obese mice by leptin injection, whereas serum NEFA was unaffected in the lean mice but reduced in the obese mice. Serum TG were reduced by leptin only in the obese mice.

Effect of acute leptin administration on lipogenesis in liver, BAT, and WAT. The effect of acute leptin treatment on lipogenic rates in liver, WAT, and BAT is shown in Fig. 1. Lipogenesis experiments were carried out on three separate occasions, and to account for any variation between experimental days, results are expressed as a percentage of the mean value for untreated lean mice on that day. Lipogenesis in the GTG mice was increased in liver, unchanged in WAT, and decreased in BAT. Leptin treatment reduced the lipogenic rate significantly in both liver and WAT in the lean mice. There was a slight increase in BAT lipogenesis, but this did not reach significance. In the obese mice, leptin produced similar reductions in lipogenesis in liver and WAT, whereas BAT lipogenesis increased in the leptin-treated groups (Table 2) and was the same for both lean and obese mice. In the untreated mice, the obese group had leptin levels that were threefold greater than those of the lean mice, as well as significantly higher insulin, glucose, NEFA, and TG than lean mice. Serum insulin levels in lean mice were unaffected by leptin treatment, but there was a significant reduction in insulin levels in the obese mice although they remained hyperinsulinemic. Serum glucose was unchanged in both lean and obese mice by leptin injection, whereas serum NEFA was unaffected in the lean mice but reduced in the obese mice. Serum TG were reduced by leptin only in the obese mice.

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WAT, but these only reached significance in WAT. There was no change in lipogenesis in BAT in obese mice.

Effect of acute leptin administration on PDHCa in heart, liver, QM, WAT, and BAT. PDHCa was decreased significantly in heart of obese mice, with a small but nonsignificant decrease in QM (Fig. 2, A and B). Liver PDHCa was increased in the obese mice, whereas WAT and BAT PDHCa was similar in lean and obese mice (Fig. 2, C, D, and E). Leptin treatment decreased PDHCa by 20–30% in all tissues tested in the lean mice. In the obese mice, similar reductions were seen in heart and skeletal muscle, but there was no effect in liver, WAT, or BAT.

Effect of acute leptin administration on TG and glycogen content of muscle and liver. Hepatic TG content was significantly greater in the obese mice, and there was a trend for muscle TG also to be higher. Glycogen content was similar in the lean and obese mice in both tissues. In lean mice, leptin had no effect on either muscle or liver glycogen content or liver TG content, but it reduced muscle TG content (Table 3). In the obese mice, significant reductions were seen in glycogen content of both liver and muscle, but there was no effect on TG content in either tissue.

DISCUSSION

These studies show that a single dose of leptin has significant effects on glucose and lipid metabolism in both lean and GTG-obese mice, with differences being seen in both the magnitude and direction of these responses between the lean and obese states. In the lean mice, reductions in the rate of lipogenesis were seen in liver and WAT, and reductions in glucose oxidation as determined by PDHCa were seen in all tissues tested. This was accompanied by reduction of muscle but not liver TG stores, with no change in muscle or liver glycogen stores. In the obese mice, where rates of lipogenesis were altered in a tissue-specific fashion, as previously described (21), leptin-induced reductions were similar to those seen in the lean mice. The tissue-specific changes in PDHCa seen in the obese mice were also as described previously (22), but leptin had an effect on PDHCa only in muscle tissue, not in liver or adipose tissue. There were greater reductions in both hepatic and muscle glycogen content but no significant change in hepatic or muscle TG content. Meanwhile, reductions were seen in serum insulin that were not seen in lean mice.

Reductions in lipogenesis, decreased tissue TG content, and decreased glucose oxidation are consistent with the hypothesis that leptin modulates energy homeostasis by directing lipid away from storage to oxidation, as suggested by in vitro studies (26). Increased fatty acid oxidation inhibits glucose oxidation by way of the glucose/fatty acid cycle (28), and we have previously shown that PDHCa in different tissues in these mice is dependent on the availability of fatty acids as an energy source (4). It is interesting that acute leptin administration was able to produce significant reductions in PDHCa in skeletal muscle in both lean and obese mice, as PDHCa is less responsive to short-term nutritional changes than in others (3). This is consistent with an acute effect of leptin mobilizing intramyocellular TG stores (9) rather than leptin having an effect on adipose tissue. In the lean mice in this study, the reduction in muscle TG content with no change in circulating NEFA is consistent with a local leptin effect.

Reductions in glycogen stores were seen in obese but not lean mice. The maintenance of hepatic glycogen content, but it reduced muscle TG content (Table 3). In
content in lean mice may be explained by a reduction in PDHCa, resulting in less glucose oxidation and a lowering of the demand for glycogen breakdown. Second, such an effect may allow glycogen synthesis from available glucose, which could compensate for any leptin-induced changes in glycolysis. This would result in no net change in glycogen content or in plasma glucose levels. In the obese mice, there was no leptin-induced inhibition of the elevated hepatic PDHCa, and therefore glucose could still be oxidized, with no sparing (as in lean mice) to allow glycogen synthesis to compensate for an increase in glycolysis. In the muscle of the obese mice, glycogenolysis was not matched by an increase in glycogen synthesis, possibly because of a combination of the leptin-induced fall in plasma insulin and insulin resistance. Chronic leptin treatment is reported to decrease hepatic glycogen content in ob/ob (15, 21) but not lean mice (21). Acute leptin treatment decreases hepatic glycogen in lean mice while increasing muscle glycogen content (4), and whole body glycogen synthesis in rats is reported to be unchanged by leptin infusion (32). In vitro studies also show variable effects on glycogen synthesis in skeletal muscle (1, 13, 26) and adipocytes (25). These mixed reports show that leptin effects on glucose metabolism are dependent on both size and duration of the leptin dose and the degree of leptin or insulin resistance in the animal model being studied, thus making comparison of studies difficult.

Reports of leptin effects on lipogenesis and lipolysis are also mixed depending on whether studies are done in vivo, in vitro, or under basal or insulin-stimulated conditions (12, 15, 23, 25, 26, 34). Lipolysis was not measured in this study, but if there is increased fatty acid oxidation, this suggests indirectly an increase in lipolysis. In this acute study, alterations in lipogenic and lipolytic rates did not result in changes in tissue

Table 3. Effect of an acute leptin injection on muscle and liver TG and glycogen content in lean and GTG-obese mice

<table>
<thead>
<tr>
<th>Group</th>
<th>TG Content, µmol/g wet wt</th>
<th>Glycogen Content, µmol/g wet wt</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Muscle</td>
<td>Liver</td>
</tr>
<tr>
<td>CON + Lp</td>
<td>29.5 ± 4.5</td>
<td>33.4 ± 2.4</td>
</tr>
<tr>
<td>CON − Lp</td>
<td>48.1 ± 10.9*</td>
<td>31.1 ± 2.9</td>
</tr>
<tr>
<td>GTG + Lp</td>
<td>63.1 ± 9.1§</td>
<td>140.1 ± 9.1§</td>
</tr>
<tr>
<td>GTG − Lp</td>
<td>63.0 ± 8.9</td>
<td>133.7 ± 10.4‡</td>
</tr>
</tbody>
</table>

Values are means ± SE for >10 mice. Leptin injection was 25 µg/mouse. *P < 0.05; †P < 0.01 for differences between treated and untreated groups; ‡P < 0.01 for differences between untreated CON and GTG; §P < 0.01 for differences between treated CON and GTG.

Fig. 2. Effect of an acute leptin injection (25 µg/mouse) on activity of pyruvate dehydrogenase complex (PDHCa) in heart (A), quadriceps muscle (B), liver (C), WAT (D), and BAT (E) of lean and obese mice. Mice were killed 2 h after leptin injection. Values are means ± SE of 8–12 mice/group. PDHCa is expressed per unit citrate synthase to correct for any differences in recovery or purity of mitochondrial extracts. *P < 0.05 for effect of leptin treatment; **P < 0.01 for effects of leptin treatment; †P < 0.05, ††P < 0.01 for effects of gold thioglycollate.
 weights or hepatic TG content, as has been reported in chronic studies with leptin (33). Whereas the fall in circulating NEFA levels in the obese mice in this study is inconsistent with a lipolytic effect, it is consistent with increased intracellular utilization of fatty acids for oxidation, whereas intracellular availability of fatty acids may not always be reflected in the circulating NEFA levels.

The decreased glucose oxidation, lipogenesis, and glycogen content seen in this study could suggest that leptin has a deleterious effect on insulin sensitivity. The association between hyperleptinemia and insulin resistance remains unresolved (30), with leptin having been shown to have both insulin-like and anti-insulin actions. Both basal and insulin-stimulated glucose utilization are reported to be improved by prior leptin infusion (16, 35), whereas other studies suggest an effect on hepatic glucose metabolism rather than on peripheral insulin action (32). We have previously shown in this model that the development of hyperinsulinemia precedes the development of hyperleptinemia, which parallels the rise in adiposity (5). A rise in leptin may be part of the process that makes these mice insulin resistant in an attempt to limit the size of the energy stores.

If leptin were increasing insulin resistance, then an increase in hyperinsulinemia could be expected and not the reduction in plasma insulin seen in the obese mice in this study. This reduction in plasma insulin is consistent with in vitro studies that have shown an inhibitory effect of leptin on islet insulin secretion (10, 17, 27, 31). The TG content of islets is an important modulator of insulin secretion (19), and leptin has been shown to deplete this (33) and increase the expression of enzymes involved in fatty acid oxidation (40), suggesting a possible mechanism for the reduced hyperinsulinemia in the obese mice.

Several factors may have contributed to the differences in the responses of the lean and obese mice to leptin in this study. First, there was a greater percent increase above basal endogenous plasma leptin levels in the lean mice than in the obese mice after leptin injection. The same dose of leptin was given to both groups on the assumption that the dose given would result in high serum levels in which any differences in the contribution of endogenous leptin would be insignificant. However, the plasma levels at the 2-h time point were identical, being sixfold higher than endogenous levels in lean mice and twofold higher in obese mice. This suggests either greater suppression of endogenous leptin secretion in the obese animals or an increased rate of clearance in the obese mice. Human studies have not found any obesity-induced changes in clearance (18), but no studies of clearance rates in mice have been reported. Studies of the effects of exogenous leptin treatment on ob mRNA expression are few, with leptin able to downregulate ob mRNA expression in the ob/ob mice but having no effect on expression in rat adipocytes in vitro (36). A recent study suggests that prolonged plasma half-life of leptin may be due to increased presence of leptin receptors in peripheral tissues, or to an increase in the ability to bind to plasma carrier-proteins (37). Whatever the reason, the obese mice in this study were receiving less of an increase in their circulating leptin levels, and this may explain the reduced magnitude of some of the metabolic responses. Because normal endogenous leptin levels are already elevated in the obese mice, leptin resistance may further diminish the response to acute increases in leptin levels.

A further contributory factor may have been that GTG-obese mice are insulin resistant, and this state combined with the leptin-induced reduction in hyperinsulinemia may have resulted in an inability to compensate for leptin effects, e.g., increased glycogen synthesis to balance leptin's effects on glycogenolysis.

Third, whereas changes seen in the obese mice suggest that hypothalamic leptin receptors are not necessary for leptin effects on glucose and lipid metabolism, the lack of hypothalamic leptin receptors in the GTG mice may have altered some of the responses in this study. Many of the reported direct in vitro effects of leptin may be obtained in vivo by intracerebroventricular injection of leptin when there is no increase in circulating leptin levels (8, 16). This suggests that both central and peripheral leptin receptors are important modulators of leptin's metabolic actions. Leptin receptors have now been identified in other parts of the brain (24), and these receptors, which may not be affected by the GTG injection, may have an indirect modulatory effect on leptin's effects in peripheral tissues. It is also possible that known GTG effects on the hypothalamic-pituitary-adrenal axis may contribute to the different responses.

These studies show that acute artificial elevation of circulating leptin inhibits lipogenesis and inhibits glucose oxidation by reducing the activity of PDHC, suggesting a stimulatory effect on fatty acid oxidation. These results are consistent with a role for leptin in the regulation of fat stores by increasing fat utilization and decreasing fat synthesis.

This study was supported by a project grant from the National Health and Medical Research Council of Australia.

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Received 7 December 1998; accepted in final form 4 May 1999.

REFERENCES


