Leptin produces anorexia and weight loss without inducing an acute phase response or protein wasting

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Kaibara, Atsushi, Armin Moshyedi, Troy Auffenberg, Amer Abouhamze, Edward M. Copeland III, Satya Kalra, and Lyle L. Moldawer. Leptin produces anorexia and weight loss without inducing an acute phase response or protein wasting. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R1518–R1525, 1998.—The ob gene product leptin is known to produce anorexia and loss of body fat when chronically administered to both lean and genetically obese mice. The current study was undertaken to examine whether administration of recombinant leptin in quantities sufficient to produce decreases in food intake and body weight and alterations in body composition would elicit either an hepatic acute phase protein response or preferential loss of carcass lean tissue. Mice were administered increasing quantities of recombinant human leptin or human tumor necrosis factor-α as a positive control. Although leptin (at 10 ng/kg body wt) produced significant anorexia and weight loss (both P < 0.05), human leptin administration did not appear to induce an hepatic acute phase protein response in either lean or genetically obese mice, as determined by protein synthetic rates in the liver or changes in the plasma concentration of the murine acute phase protein reactants, amyloid A, amyloid P, or seromucoid (α2-acid glycoprotein). In addition, human leptin administration did not induce a loss of fat-free dry mass (protein) in lean or obese animals. The findings suggest that at doses adequate to alter food intake and body weight leptin is not a significant inducer of the hepatic acute phase response nor does leptin promote the preferential loss of somatic protein characteristic of a chronic inflammatory process.

leptin; human tumor necrosis factor; mouse; liver; cachexia

WEIGHT LOSS, ANOREXIA, and a dissolution of carcass protein and fat accompanies most chronic inflammatory processes. Although anorexia alone can explain much of the weight loss that accompanies chronic inflammation, losses of lean tissue and body fat frequently exceed that explained solely by reduced food intake. Inflammation-mediated weight loss is often associated with increases in resting energy expenditure, alterations in intermediary substrate metabolism, and changes in somatic and visceral protein synthesis.

This wasting diatheses is presumed to be mediated by several proinflammatory cytokines, including IL-1, tumor necrosis factor-α (TNF-α), and IL-6. Blockade of IL-1 or IL-6 in turpentine-induced myositis attenuates weight loss and anorexia (12, 32, 33), whereas blockade of TNF-α, IL-1, or IL-6 diminishes the cachexia associated with some actively growing tumors (11, 23, 39). Administration of recombinant TNF-α or IL-1 can also replicate much of the weight loss and acute phase protein responses seen in inflammation (9, 27, 41).

Leptin is a 16-kDa protein expressed predominantly in adipose tissue. When administered to both lean and genetically obese mice, leptin has been shown to produce anorexia and loss of body fat without apparent changes in skeletal muscle protein content (35, 45). However, several aspects of leptin biology have implicated it in the myriad of host responses to inflammation. First, Grunfeld and colleagues (14) demonstrated that the leptin gene is upregulated in adipose tissue in response to lipopolysaccharide, TNF-α, or IL-1. Similarly, administration of several proinflammatory cytokines, including IL-1 and TNF-α, as well as corticosteroids, induces an increased leptin mRNA and protein response (18, 34, 37). We have recently observed that plasma leptin concentrations and adipose tissue leptin mRNA levels are increased in gram-negative peritonitis (28a) where anorexia and wasting occur. Grunfeld et al. (14) recently proposed that inflammation-induced upregulation of TNF- and IL-1 may therefore lead to anorexia and weight loss via increased leptin production (14).

More recent data suggest that leptin may act directly on tissues outside of the central nervous system (CNS) and exert biological actions distinct from its effects on satiety or sexual maturation. For example, Liu and colleagues (22) reported that leptin inhibited insulin-stimulated glycogen synthesis in isolated soleus muscle preparations (22), whereas Muuo et al. (30) observed that leptin stimulated skeletal muscle fatty acid oxidation. These findings demonstrate that skeletal muscle carbohydrate and fat metabolism are altered by leptin, whereas protein metabolism was not explored. Leptin has also been implicated as a mediator of insulin resistance in adipocytes (29). Similarly, leptin appears to act through distinct receptors in the adrenal gland to suppress cortisol release (1).

Controversy exists over whether functional leptin receptors exist in the liver. Whereas Emilsson et al. (6) and Ghirardi et al. (13) identified functional Ob receptor mRNA (by RT-PCR) in livers, others, including Wang et al. (43) and Fei et al. (8), were unable to do so. Surprisingly, Fei et al. (8) described a novel leptin receptor isoform in livers of rats. In a recent report, Wang et al. (44) have observed that HepG2 cell lines expressing functional Ob receptors respond to leptin with an IL-6 receptor-like signaling that includes the activation of signal transducers and activators of transcription (STAT) proteins, induction of acute-phase plasma proteins, and synergism with IL-1 and TNF-α.
period, food intake and body weight were monitored daily. During this period, animals were housed four per cage in plastic shoe boxes with pine shavings in a light (0700–1900)- and temperature-controlled room (20–24°C) for a period of 7 days. During this period 45 mice received, at 1700, an intraperitoneal injection (200 µl) of recombinant human leptin (1.0 or 10 mg/kg body wt) and the third group received recombinant human TNF-α (0.25 mg/kg body wt). Two additional control groups received sham intraperitoneal injections of PBS with 0.1% mouse serum (200 µl), but the animals were either allowed to consume their food freely or were pair-fed equivalent quantities of food as consumed by animals receiving 10 mg/kg body wt leptin.

The intraperitoneal injections of leptin, TNF-α, or PBS were repeated on a daily basis for the next 4 days. Each day, the injections were given at 1700. On each morning, food intake and body weight were recorded.

On day 5, at 0900, mice were gently restrained and received an intraperitoneal injection of 1.5 µmol/g body wt L-phenylalanine containing 0.25 µCi/g body wt L-[U-14C]phenylalanine (Amersham). Exactly 10 min later, the mice were killed by cervical dislocation. The thoracic cavity was opened, and the animals were bled by cardiac puncture. Serum was separated by centrifugation and stored at −70°C. The liver and gastrocnemius muscle were removed, tared, and frozen immediately in liquid nitrogen. The mouse carcasses were eviscerated, hair was removed, and the animal carcasses were frozen at −20°C for analysis.

Finally, in the 12-day study (study 3), an additional 32 female C57BL/6 and 64 ob/ob mice were allowed to equilibrate for 5–7 days, during which time body weight and food intake were monitored daily. At 1700, mice were randomized to one of three treatment groups; one group received intraperitoneal injections (200 µl) of recombinant human leptin at the highest dose (10 mg/kg body wt). Two additional groups received sham intraperitoneal injections of PBS with 0.1% mouse serum (200 µl). The animals were either allowed to consume their food freely or were pair-fed equivalent quantities of food as consumed by animals receiving 10 mg/kg body wt of leptin. At time of death on day 12, the mouse carcasses were eviscerated, hair was removed, and the animal carcasses were frozen at −20°C for analysis.

Analytic procedures. Serum was analyzed for total protein, albumin, triglycerides, and the concentration of three positive acute phase reactant proteins, amyloid A, amyloid P, and seromucoid. Triglycerides, total protein, and albumin concentrations were determined colorimetrically using commercial reagents (Sigma Chemical). Seromucoid fraction, which is predominantly α1-acid glycoprotein, was obtained by sequential precipitation of serum in 0.6 M perchlorate and 2% phosphotungstic acid, as described by Hellerstein et al. (17). Amyloid P was determined by rocket immunoelectrophoresis (12). Five microliters of serum were added to circular wells punched into a 1% agarose gel containing 0.5% rabbit anti murine serum amyloid P (Cal-Biochem, Santa Clara, CA) and electrophoresed at 200 V for 18 h at 4°C in Tris-barbital (pH 8.6) buffer. Immune precipitates were visualized by staining the gels for 15 min with 0.2% Coomassie brilliant blue. The quantity of amyloid P was determined from the height of the rockets compared with a commercial standard. Amyloid A protein was determined with a commercially available enzyme-linked immunosorbent assay (BioSource International, Camarillo, CA).
Car cass water, fat, and fat-free dry mass were determined gravimetrically (9). Eviscerated carcasses were weighed directly after killing the mice. Subsequently, the carcasses were frozen in liquid nitrogen and pulverized with solid carbon dioxide in a commercial blender. Pulverized carcasses were then dried to a constant mass at 80°C. Lipid content was determined by sequential chloroform-methanol (1:1), ethanol-acetone (1:1), and petroleum ether extractions. Fat-free dry mass was used as an estimate of carcass protein content (12).

Fractional rates of protein synthesis in the liver, muscle, and total serum protein were estimated from the incorporation of [14C]phenylalanine into acid-precipitated protein, as originally described by Garlick et al. (10). Approximately 200 mg of frozen liver or muscle or 200 µl of serum were homogenized in five weight:volume s of 1.2 M perchlorate, and the protein precipitate was washed at least three times. Acid-precipitated protein was solubilized with 2 N sodium hydroxide, and aliquots were neutralized with glacial acetic acid and set aside for protein content and total 14C radioactivity.

The protein-free supernatants from liver and muscle were neutralized with normal KOH and used for measurement of free phenylalanine specific radioactivity (10). Determination of the free [14C]phenylalanine specific radioactivity involved its enzymatic conversion to β-phenethylamine with phenylalanine decarboxylase. β-Phenethylamine was extracted from other constituents by sequential extraction into basic chloroform-n-haptane (1:3) and then into 0.01 M sulfuric acid. β-Phenethylamine concentration was determined fluorometrically, and total 14C radioactivity was determined by liquid scintillation spectrometry.

Fractional rate of protein synthesis (k_s) in liver, gastrocnemius muscle, and total plasma protein was calculated from the specific radioactivity of phenylalanine in protein (S_p; dpm/µmol) obtained at death at 10 min and the mean specific radioactivity of free phenylalanine in the tissue (S_f; dpm/µmol) between 0 and 10 min. It was assumed that mouse protein comprised 5% phenylalanine by weight, and all of the radioactivity in the acid-precipitated protein was due to [14C]phenylalanine (28). The value of S_f at 0 min was assumed to be equal to the specific radioactivity of the infusate, because a flooding dose was employed. For total plasma protein synthesis, free phenylalanine specific radioactivity in the liver was used for S_f. Absolute rates of total liver protein synthesis (mg/day) were calculated from the product of the fractional rate of synthesis and total liver protein content.

Statistical analyses. All data were expressed as means ± SE. Differences among the treatment groups were analyzed by ANOVA using a commercial statistics package (Statview 412 [Abacus Concepts, Berkeley, CA] or SigmaStat [Sandel Scientific, Santa Clara, CA]) or a Macintosh LCII personal computer. Post hoc comparisons among the study groups were performed with the Student-Newman-Keuls multiple-range test.

RESULTS

Twenty-four-hour study. Mice that received human leptin showed decreases in food intake and body weight (Fig. 1A), although in the acute 24-h study, the decreases did not reach statistical significance. However, at the highest doses of human leptin, food intake declined 24%. In contrast, 0.25 mg/kg body wt of TNF-α significantly decreased food intake by 42% (P < 0.02) and produced weight loss of 0.6 ± 0.2 g (P < 0.002).

Similarly, none of the mice treated with the human leptin preparation exhibited any significant increase in hepatic acute phase protein concentrations over the first 24 h (Table 1). Administration of 0.1, 1, or 10 mg/kg body wt of human leptin had no effect on either seromucoid, amyloid A, or amyloid P concentrations. However, mice that received TNF-α significantly increased their plasma concentrations of seromucoid (P < 0.0001), amyloid A (P < 0.0001), and amyloid P (P < 0.0001).

Five-day study. Mice receiving leptin showed significant decreases in food intake (P < 0.01) and body weight (P < 0.01) over the 5-day study period (Fig. 2). The reductions in food intake (Fig. 2) were greatest in the mice receiving the highest dose of leptin. Unlike mice treated with TNF-α, which showed tolerance and decreased their food intake only on the first 2 study days, albeit not significantly, food intake remained depressed and body weight was continuously lost in

| Table 1. Serum acute phase reactants 24 h after a single injection of leptin or TNF-α |
|-----------------------------------|----------------|------------|---------|
|                                   | Seromucoid     | Amyloid A  | Amyloid P |
| 0.1 mg/kg body wt leptin          | 72.8 ± 6.4     | 14.5 ± 0.16| 3.4 ± 0.3 |
| 1.0 mg/kg body wt leptin          | 70.6 ± 3.2     | 13.6 ± 0.5 | 1.2 ± 1.2 |
| 10 mg/kg body wt leptin           | 74.4 ± 2.8     | 13.4 ± 0.3 | <1       |
| 0.25 mg/kg TNF-α                  | 125.8 ± 3.8*   | 27.3 ± 0.11*| 15.6 ± 2.2*|
| Freely fed                        | 79.2 ± 3.8     | 16.0 ± 0.14| 3.0 ± 3.0 |

Values are means ± SE in µg/ml (n = 9/group). TNF, tumor necrosis factor. *P < 0.05 vs. freely fed animals by 1-way ANOVA and Student-Newman-Keuls multiple-range test.
leptin-treated mice over the entire study period. Mice that received 10 mg/kg leptin continued to decrease food intake until the end of the study. Food intakes significantly differed after day 3 between mice receiving the higher dose of leptin (10 mg/kg body wt) and freely fed controls (P < 0.05). Food intake was also significantly reduced in the mice receiving 1.0 mg/kg body wt leptin (P < 0.05). The cumulative food intake was significantly reduced (both P < 0.05) by 13 and 19% in mice receiving 1.0 and 10 mg/kg body wt leptin, respectively, compared with the freely fed control group receiving PBS over the first 5 days.

Mice that received 10 mg/kg per day lost significant weight after 3 days compared with the freely fed group (P < 0.05) (Fig. 2). Mice that received the lower dose of leptin did not exhibit a significant change in body weight, whereas freely fed mice continued to gain body weight over the study period. Mice pair-fed quantities of food consumed by mice treated with 10 mg/kg body wt of leptin also lost body weight, but the differences between pair-fed and leptin-treated animals was not statistically significant. Despite significant losses in body weight after 5 days of leptin treatment, changes in carcass fat and fat-free dry weight were not statistically significant (Table 2).

In contrast to leptin, mice that received TNF-α for 5 days had significantly increased liver weight (1.21 ± 0.06 vs. 0.89 ± 0.05 g) compared with the freely fed group (P < 0.0001). The mice that received TNF-α also had significantly decreased albumin (P < 0.0002) and increased triglyceride (P < 0.05) and acute phase protein (seromucoid fraction of plasma protein, P < 0.0001; amyloid A, P < 0.05; and amyloid P, P < 0.0001) concentrations (Table 3). In contrast, leptin-treated mice had no significant change in any of these parameters.

Protein kinetic measures are presented in Table 4. Total liver protein and plasma protein fractional synthesis rates were increased 70 and 64%, respectively, in mice treated with TNF-α (both P < 0.05). Muscle fractional synthesis rate was also significantly decreased with pair-fed groups compared with freely fed animals (P < 0.03). However, there were no significant differences in fractional protein synthesis rates between leptin-treated and freely fed or pair-fed animals.

Twelve-day study. Administration of 10 mg/kg body wt of human leptin produced significant declines in food intake and body weight in both lean and genetically obese mice over the 12 days (Table 5). No evidence of tachyphylaxis or tolerance to the human leptin preparations was observed; the reductions in food intake on day 12 were similar to that seen in the first few days. Genetically obese mice lost approximately 32% of their total body fat during that period (P < 0.01), whereas fat-free carcass dry weight was unchanged. Pair-fed animals also lost comparable amounts of body fat as leptin-treated animals. Normal mice treated with leptin lost modest amounts of body fat and no carcass protein.

As observed after 5 days of leptin administration, there was no evidence of an hepatic acute phase protein response in either lean or genetically obese mice given leptin for 12 days (Table 6). However, leptin administration for 12 days significantly reduced serum triglyceride levels in both lean and genetically obese animals. Serum amyloid A, amyloid P, and seromucoid levels were not affected by leptin administration. In fact,

Table 2. Carcass composition analysis after 5 days of leptin administration

<table>
<thead>
<tr>
<th>Carcass Composition Analysis after 5 days of leptin administration</th>
<th>Body Weight, g</th>
<th>Carcass Wet</th>
<th>Body Weight, g</th>
<th>Carcass Fat</th>
<th>Body Weight, g</th>
<th>Carcass Fat-Free</th>
<th>Dry Weight, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0 mg/kg body wt leptin</td>
<td>18.3 ± 0.2*</td>
<td>12.1 ± 0.1</td>
<td>0.66 ± 0.085</td>
<td>66.0 ± 1.0</td>
<td>2.53 ± 0.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 mg/kg body wt leptin</td>
<td>17.7 ± 0.2*</td>
<td>12.0 ± 0.1</td>
<td>0.65 ± 0.082</td>
<td>66.5 ± 1.1</td>
<td>2.72 ± 0.61</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.25 mg/kg TNF-α</td>
<td>19.4 ± 0.3</td>
<td>11.8 ± 0.1</td>
<td>0.76 ± 0.095</td>
<td>66.0 ± 1.2</td>
<td>2.23 ± 0.35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pair fed</td>
<td>17.5 ± 0.4*</td>
<td>11.9 ± 0.2</td>
<td>0.65 ± 0.066</td>
<td>67.3 ± 0.6</td>
<td>2.50 ± 0.43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Freely fed</td>
<td>19.0 ± 0.3</td>
<td>12.4 ± 0.1</td>
<td>0.75 ± 0.075</td>
<td>67.2 ± 0.9</td>
<td>2.67 ± 0.37</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 12/group). *P < 0.05 vs. freely fed animals by 1-way ANOVA and Student-Newman-Keuls multiple-range test.
levels in all of the animals were equivalent to freely fed animals. Surprisingly, seromucoid levels tended to be higher in obese mice than in lean animals, and food restriction in these animals (pair-feeding) actually increased seromucoid levels compared with leptin-treated animals (P < 0.05). Surprisingly, three of the eight ob/ob mice pair-fed to the leptin-treated animals also had a serum amyloid A response, although as a group the differences were not significant.

### DISCUSSION

Recent interest has focused on the role of leptin in the regulation of food intake and body weight in both health and disease (21). Leptin is produced primarily by adipocytes, and circulating levels in humans and rodents appear to be closely related to the quantity of body fat (5, 25). However, there is also accumulating evidence that plasma leptin concentrations may vary in response to feeding and to changes in circulating hormone concentrations. For example, Saladin and colleagues (36) reported diurnal variations in leptin gene expression in the mouse; food intake and chronic insulin administration also increased leptin mRNA levels (16, 19, 36). Conversely, fasting is associated with decreases in both leptin mRNA and plasma protein levels (16, 24, 26, 42). Not surprisingly, hormones and humoral factors produced during inflammation have also been shown to regulate leptin gene expression. For example, the corticosteroid dexamethasone increased adipocyte leptin expression four- to eightfold within 7 h (31, 34, 38). Grunfeld et al. (14) recently reported that administration of the gram-negative bacterial product lipopolysaccharide to hamsters also increased adipose tissue leptin mRNA, and there was a strong inverse associative relationship between leptin mRNA levels and food intake. Furthermore, administration of recombinant TNF-α or IL-1 in quantities sufficient to reduce food intake increased both leptin mRNA levels (50–100%) and plasma concentrations of leptin protein (37). The findings suggest that increased leptin levels may be a component of the host response and contribute to the anorexia that accompanies acute and chronic inflammation.

In addition to altering food intake, leptin administration also increases energy expenditure in obese mice (15, 35) and restores fertility (2). Although such responses may be directly linked to CNS processes also involved in food intake, the distribution of leptin receptors outside of the CNS suggests that leptin may have additional functions independent of the CNS. Other investigators have demonstrated that mRNA for both truncated and functional, full-length leptin receptors can also be found in liver and lung (3, 20, 40, 44), although the levels of the functional receptor are much lower than the levels of the truncated receptor.

In skeletal muscle, leptin binding to the functional Ob receptor inhibited insulin-mediated glycogen synthesis (22), increased fatty acid oxidation, and decreased triglyceride synthesis (30). Its effects on protein metabolism were not examined. Approximately 5% of the leptin receptors on hepatoma cells are the functional form and are responsible for the attenuation of insulin-mediated suppression of gluconeogenesis by leptin in human hepatocytes (4). In a recent report, Wang et al. (44) have observed that HepG2 cell lines expressing functional Ob receptors responded to leptin with an IL-6 receptor-like signaling that included the activation of STAT proteins, induction of acute-phase plasma proteins, and synergism with IL-1 and TNF-α. How-

### Table 3. Plasma protein levels in mice treated with recombinant proteins

<table>
<thead>
<tr>
<th>Protein</th>
<th>Total Protein, mg/ml</th>
<th>Albumin, g/l</th>
<th>Triglycerides, mg/dl</th>
<th>Seromucoid, µg/ml</th>
<th>Amyloid A, µg/ml</th>
<th>Amyloid P, µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0 mg/kg body wt leptin</td>
<td>45.5 ± 0.6</td>
<td>32.4 ± 0.6</td>
<td>120 ± 9</td>
<td>95.4 ± 4.2</td>
<td>7.2 ± 0.2</td>
<td>1.2 ± 1.2</td>
</tr>
<tr>
<td>10 mg/kg body wt leptin</td>
<td>45.7 ± 1.0</td>
<td>32.3 ± 0.9</td>
<td>102 ± 6</td>
<td>101.4 ± 3.5</td>
<td>7.1 ± 0.2</td>
<td>1.0 ± 0.6</td>
</tr>
<tr>
<td>0.25 mg/kg TNF-α</td>
<td>44.7 ± 1.0</td>
<td>28.9 ± 0.7*</td>
<td>122 ± 4*</td>
<td>258.0 ± 11.0*</td>
<td>14.6 ± 0.5*</td>
<td>26.6 ± 2.2*</td>
</tr>
<tr>
<td>Pair fed</td>
<td>46.9 ± 0.5</td>
<td>33.0 ± 0.7</td>
<td>105 ± 3</td>
<td>93.0 ± 2.7</td>
<td>7.0 ± 0.2</td>
<td>0.3 ± 0.2</td>
</tr>
<tr>
<td>Freely fed</td>
<td>47.1 ± 1.0</td>
<td>32.9 ± 0.6</td>
<td>109 ± 6</td>
<td>102.4 ± 4.0</td>
<td>7.4 ± 0.5</td>
<td>2.2 ± 1.6</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 12/group). *P < 0.05 vs. freely fed animals by 1-way ANOVA and Student-Newman-Keuls multiple-range test.

### Table 4. Protein kinetic parameters

<table>
<thead>
<tr>
<th>Protein</th>
<th>Liver FSR, %/day</th>
<th>Total Liver Synthesis, mg/day</th>
<th>Plasma FSR, %/day</th>
<th>Muscle FSR, %/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0 mg/kg body wt leptin</td>
<td>66.3 ± 12.5</td>
<td>43.4 ± 7.1</td>
<td>29.4 ± 3.9</td>
<td>10.8 ± 0.7</td>
</tr>
<tr>
<td>10 mg/kg body wt leptin</td>
<td>73.2 ± 10.9</td>
<td>45.6 ± 4.4</td>
<td>24.7 ± 3.9</td>
<td>11.2 ± 1.1</td>
</tr>
<tr>
<td>0.25 mg/kg TNF-α</td>
<td>118.4 ± 36.8</td>
<td>93.8 ± 27.0*</td>
<td>37.4 ± 5.7*</td>
<td>12.4 ± 0.9</td>
</tr>
<tr>
<td>Pair fed</td>
<td>76.1 ± 10.7</td>
<td>50.1 ± 6.6</td>
<td>23.6 ± 3.7</td>
<td>8.8 ± 1.0*</td>
</tr>
<tr>
<td>Freely fed</td>
<td>72.2 ± 10.4</td>
<td>55.2 ± 7.1</td>
<td>22.7 ± 2.9</td>
<td>11.8 ± 0.7</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 12/group). FSR, fractional synthesis rate, % protein synthesized per day. *P < 0.05 vs. freely fed animals by 1-way ANOVA and Student-Newman-Keuls multiple-range test.

### Table 5. Changes in food intake and body weight in lean and obese mice treated for 12 days with 10 mg/kg body wt of recombinant human leptin

<table>
<thead>
<tr>
<th>Protein</th>
<th>Lean C57BL/6</th>
<th>ob/ob</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food intake, g/day</td>
<td>-6.4 ± 1.2*</td>
<td>2.2 ± 0.2*</td>
</tr>
<tr>
<td>Body weight change, g</td>
<td>-17.4 ± 0.5*</td>
<td></td>
</tr>
<tr>
<td>Pair fed</td>
<td>-8.3 ± 0.9*</td>
<td>2.2</td>
</tr>
<tr>
<td>Freely fed</td>
<td>3.5 ± 0.1</td>
<td>-1.1 ± 0.8</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 8 for C57BL/6 and 16 for ob/ob). *P < 0.05 vs. freely fed. Data were analyzed by 2-way ANOVA and post hoc comparisons were performed using Student-Newman-Keuls multiple-range test.
ever, HepG2 cells not expressing the receptor were resistant to leptin-mediated IL-6-like responses. It is still unresolved whether animals respond to leptin with a skeletal muscle protein or hepatic protein response.

The present study was therefore undertaken to examine whether administration of leptin to mice in quantities sufficient to produce anorexia and weight loss would also induce an hepatic acute phase protein response and skeletal muscle protein catabolism similar to that seen in inflammation. The results were unequivocal. Administration of up to 10 mg/kg body wt of human leptin to both the lean and genetically obese mouse resulted in significant anorexia and weight loss, but had no effect at either 24 h, 5 days, or 12 days on any aspect of hepatic acute phase protein synthesis or carcass protein content. Total plasma or hepatic protein synthesis was unaffected, as were the concentrations of the positive murine acute phase protein reactants, amyloid A, amyloid P, and seromucoid.

There was also no evidence that chronic leptin administration produced any preferential loss of lean tissue. Both lean and genetically obese mice treated with recombinant human leptin for periods up to 12 days had no significant change in carcass lean tissue, as measured by fat-free dry weight. These findings are consistent with earlier observations of Pelleymounter et al. (35) and Halaas et al. (15), who also reported a sparing of lean tissue with leptin administration.

Unfortunately, we were unable to show a significant loss of body fat in lean mice treated with leptin for either 5 or 12 days, although reductions in food intake and body weight were significant. In this regard, the results differ somewhat from the earlier findings of Pelleymounter et al. (35), who observed significant losses of body fat in lean animals. However, those investigators reported much more modest losses of body fat in lean animals compared with genetically obese animals (15, 35), a finding similar to that observed here. The explanation is probably methodological, because the lean juvenile mice (C57BL/6) employed in this study contained only 5–8% of body weight as fat. The gravimetric methodology employed and its inherent variance could not discriminate the small amounts of body fat presumably lost in these young animals. In contrast, in the obese mice treated with the same doses of leptin, the animals lost quantities of body fat over the 12-day period that were easily discriminated with the gravimetric methodologies.

### Perspectives

Leptin appears to be an endocrine factor whose primary functions include satiety and sexual maturation. The administration of leptin to overweight adults has been promulgated as a means to reduce dietary intake and promote weight loss. Although there are a variety of other humoral factors that produce anorexia when administered to healthy animals, including TNF-α, IL-1, IL-6, ciliary neurotrophic factor (CNTF), and leukemia inhibitory factor, their acute and chronic administration has been associated with adverse side effects primarily linked to their proinflammatory properties. Although in vitro studies have shown that leptin can also act directly on a variety of peripheral tissues, including skeletal muscle, liver, adipocytes, pancreas, and the adrenals, the studies presented here clearly demonstrate that, when administered to healthy animals in quantities sufficient to induce anorexia and weight loss, leptin is nearly devoid of any proinflammatory properties. The findings also suggest that leptin administration does not appear to regulate protein balance. The losses in body weight in both lean and genetically obese mice and the losses in body fat in the obese animals could be explained entirely by the associated anorexia. In this regard, administering leptin as a means of reducing food intake in overweight individuals is unlikely to be associated with any adverse protein metabolic effects, consistent with inflammation. On a per gram basis, leptin is more weakly anorexigenic than the proinflammatory cytokines TNF-α, IL-1, and CNTF. These cytokines produce anorexia of greater magnitude with far lower doses (7, 9, 27). However, tachyphylaxis to leptin does not develop as readily as to TNF-α. Rather, leptin appears to act specifically on food intake and energy balance without any significant impact on skeletal muscle and hepatic protein metabolism.

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REFERENCES


