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 mg/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 serumucoid (α1-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 produces anorexia and weight loss without inducing an acute phase response or protein wasting. 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.

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 Muoio 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 Ghilardi 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.
However, HepG2 cells not expressing the receptor were resistant to leptin-mediated responses. Because leptin may act on peripheral tissues directly, such as the liver and skeletal muscle, and may be involved in some aspects of insulin resistance, the present studies were undertaken to determine if systemic administration of leptin at levels sufficient to produce anorexia and weight loss would also induce an acute phase response and carcass protein losses consistent with inflammation. Studies were conducted primarily in lean mice, although genetically obese mice were also used (ob/ob) in some of the chronic studies. These latter animals produce an inactive form of leptin and are hyperphagic and obese (15). The findings suggest that leptin administration is without any significant effect on hepatic or skeletal muscle protein kinetics; rather, the weight loss and losses of body fat secondary to leptin administration can be explained primarily by the associated anorexia.

**MATERIALS AND METHODS**

Reagents. Recombinant human leptin was provided by Amgen (Thousand Oaks, CA). The recombinant protein was generated in an Escherichia coli expression system and was purified to homogeneity by ion exchange and affinity chromatography. Endotoxin content of the human leptin preparation was 0.3 IU/mg protein, respectively. Recombinant human TNF-α was obtained from Amgen, and endotoxin content was <0.5 IU/mg protein. All other reagents were obtained from Sigma Chemicals (St. Louis, MO), unless otherwise noted.

Experimental designs. Three studies were performed. The first study was an acute investigation (24 h) aimed at determining the optimal dose of recombinant human leptin in the mouse. The second study was aimed at investigating whether doses of human leptin sufficient to produce anorexia and losses of body weight also induced skeletal muscle and hepatic protein kinetic changes after 5 days. In both studies, leptin administration was compared with administration of recombinant human TNF-α, which has been previously shown to produce anorexia, weight loss, and changes in acute phase protein metabolism (6, 23, 34). The third study was aimed at examining body compositional changes in both lean and genetically obese mice administered recombinant human leptin for 12 days. The latter period was chosen because 1 and 5 days administration of leptin were inadequate to discern carcass compositional changes in lean mice.

All protocols were approved by the Institutional Animal Care and Use Committee of the University of Florida. The laboratory adheres to the Guide for the Care and Use of Laboratory Animals, as promulgated by the American Physiological Society.

**Female C57BL/6 (Charles River Breeding Laboratories, Wilmington, MA) mice weighing between 15 and 20 g and female ob/ob (Jackson Laboratories, Bar Harbor, ME) mice weighing between 30 and 50 g were used for all studies. 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, food intake and body weight were monitored daily.**

In the 24-h study (study 1), after a 7-day equilibration period, 45 mice received, at 1700, an intraperitoneal injection (200 µl) of either 0.1, 1.0, or 10 mg/kg body wt recombinant human leptin, 0.25 mg/kg body wt of human TNF-α, or PBS (pH 7.4) with 0.1% mouse serum. The recombinant proteins (human leptin and TNF-α) were diluted in PBS with 0.1% mouse serum. The following morning at 0900, 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 until analysis.

In the 5-day study (study 2), 60 female C57BL/6 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; two of the treatment groups received intraperitoneal injections (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 immunoassay (BioSource International, Camarillo, CA).
Carcass 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:volumes 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-phenylethylamine with phenylalanine decarboxylase. β-Phenylethylamine was extracted from other constituents by sequential extraction into basic chloroform-n-haptane (1:3) and then into 0.01 M sulfuric acid. β-Phenylethylamine concentration was determined fluorometrically, and total 14C radioactivity was determined by liquid scintillation spectrometry.

Fractional rate of protein synthesis (kS) in liver, gastrocnemius muscle, and total plasma protein was calculated from the specific radioactivity of phenylalanine in protein (S; dpm/µmol) obtained at death at 10 min and the mean specific radioactivity of free phenylalanine in the tissue (Sf; 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 Sf 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 Sf. 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 (Jandel Scientific, Santa Clara, CA) on a Macintosh LCIII 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. 1), 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 the PBS control (sham-treated group, freely fed) groups. *P < 0.05 vs. PBS (sham-treated group, freely fed), by 1-way ANOVA and Student-Newman-Keuls post hoc test.

<table>
<thead>
<tr>
<th>Leptin</th>
<th>0.1 mg/kg body wt leptin</th>
<th>1.0 mg/kg body wt leptin</th>
<th>10 mg/kg body wt leptin</th>
<th>0.25 mg/kg TNF-α</th>
<th>Freely fed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seromucoid</td>
<td>72.8 ± 6.4</td>
<td>70.6 ± 3.2</td>
<td>74.4 ± 2.8</td>
<td>125.8 ± 3.8*</td>
<td>79.2 ± 3.8</td>
</tr>
<tr>
<td>Amyloid A</td>
<td>14.5 ± 0.16</td>
<td>13.6 ± 0.5</td>
<td>13.4 ± 0.3</td>
<td>27.3 ± 0.11*</td>
<td>16.0 ± 0.14</td>
</tr>
<tr>
<td>Amyloid P</td>
<td>3.4 ± 3.4</td>
<td>1.2 ± 1.2</td>
<td>&lt;1</td>
<td>15.6 ± 2.2*</td>
<td>3.0 ± 3.0</td>
</tr>
</tbody>
</table>

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.
Carcass composition analysis after 5 days of leptin administration significantly differed after food intake until the end of the study. Food intakes that received 10 mg/kg leptin continued to decrease leptin-treated mice over the entire study period. Mice administered TNF-α decreased their food intake on the first 2 days of administration, the animals rapidly became tolerant to the administrations and food intake promptly returned to normal and body weight was unchanged after 5 days. Animals pair-fed (PF) equivalent quantities of food consumed by 10 mg/kg body wt leptin-administered mice lost greater, albeit not significantly, amounts of body weight (BW). *P < 0.05 vs. freely fed animals, by 1-way ANOVA and Student-Newman-Keuls post hoc test.

Figure 2. Daily food intake (A) and body weight changes (B) in mice repeatedly administered human leptin (Lep) or TNF-α for 5 days. Administration of 10 mg/kg body wt recombinant human leptin produced sustained decreases in food intake and losses in body weight. One milligram per kilogram human leptin did not produce significant decreases in food intake, but did result in a significant reduction in body weight compared with freely fed (FF) mice. Although mice administered TNF-α decreased their food intake on the first 2 days of administration, the animals rapidly became tolerant to the administrations and food intake promptly returned to normal and body weight was unchanged after 5 days. Animals pair-fed (PF) equivalent quantities of food consumed by 10 mg/kg body wt leptin-administered mice lost greater, albeit not significantly, amounts of body weight (BW). *P < 0.05 vs. freely fed animals, by 1-way ANOVA and Student-Newman-Keuls post hoc test.

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

<table>
<thead>
<tr>
<th></th>
<th>Body Weight, g</th>
<th>Carcass Wet Weight, g</th>
<th>Carcass Fat Content, g</th>
<th>Carcass % Water</th>
<th>Carcass Fat-Free 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>
</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>
</tr>
<tr>
<td>0.25 mg/kg body wt 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>
</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>
</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>
</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.
leptin mRNA, and there was a strong inverse relationship between leptin mRNA 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 Ob receptor and 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.

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

<table>
<thead>
<tr>
<th>Protein</th>
<th>1.0 mg/kg body wt leptin</th>
<th>10 mg/kg body wt leptin</th>
<th>0.25 mg/kg TNF-α</th>
<th>Pair fed</th>
<th>Freely fed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Protein, mg/ml</td>
<td>45.5 ± 0.6</td>
<td>45.7 ± 1.0</td>
<td>44.7 ± 1.0</td>
<td>46.9 ± 0.5</td>
<td>47.1 ± 1.0</td>
</tr>
<tr>
<td>Albumin, g/l</td>
<td>32.4 ± 0.6</td>
<td>32.3 ± 0.9</td>
<td>28.9 ± 0.7*</td>
<td>33.0 ± 0.7</td>
<td>32.9 ± 0.6</td>
</tr>
<tr>
<td>Triglycerides, mg/dl</td>
<td>120 ± 9</td>
<td>102 ± 6</td>
<td>122 ± 4*</td>
<td>105 ± 3</td>
<td>109 ± 6</td>
</tr>
<tr>
<td>Seromucoid, µg/ml</td>
<td>95.4 ± 4.2</td>
<td>101.4 ± 3.5</td>
<td>258.0 ± 11.0*</td>
<td>93.0 ± 2.7</td>
<td>102.4 ± 4.0</td>
</tr>
<tr>
<td>Amyloid A, µg/ml</td>
<td>7.2 ± 0.2</td>
<td>7.1 ± 0.2</td>
<td>14.6 ± 0.5*</td>
<td>7.0 ± 0.2</td>
<td>7.4 ± 0.5</td>
</tr>
<tr>
<td>Amyloid P, µg/ml</td>
<td>1.2 ± 1.2</td>
<td>1.0 ± 0.6</td>
<td>26.6 ± 2.2*</td>
<td>0.3 ± 0.2</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</th>
<th>Total Liver</th>
<th>Plasma</th>
<th>Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSR, %/day</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>Synthesis, mg/day</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>
</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>1.0 mg/kg body wt leptin</td>
<td>3.1 ± 0.1*</td>
</tr>
<tr>
<td>Body weight change, g</td>
<td>6.4 ± 1.2*</td>
<td>-6.4 ± 1.2*</td>
</tr>
<tr>
<td>Pair fed</td>
<td>2.2 ± 0.2*</td>
<td>2.2 ± 0.2*</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 thus 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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LEPTIN AND ACUTE PHASE PROTEIN RESPONSES

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