Efficacy of exogenous recombinant murine leptin in lean and obese 10- to 12-mo-old female CD-1 mice

MARY ANN PELLEYMOUNTER,1 MARY JANE CULLEN,1 DENIS HEALY,1 RANDY HECHT,2 DWIGHT WINTERS,2 AND MICHAEL MCCaleb1

1Departments of Neuroscience and 2Process Development, Amgen, Thousand Oaks, California 91320

Efficacy of exogenous recombinant murine leptin in lean and obese 10- to 12-mo-old female CD-1 mice. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R950–R959, 1998.—Leptin efficacy was compared in obese and lean female CD-1 mice. Body weights in these 10- to 12-mo-old mice ranged from 29.7 to 62.0 g, and leptin levels correlated with body weight. Mice from the lean and obese ends of the weight distribution were treated with daily peripheral leptin injections (1–100 mg/kg) for a 33-day period. The half-maximal effective doses for weight loss and fat reduction were shifted 0.5–0.7 log to the right for obese mice. Leptin was less efficacious at low doses (1–3 mg/kg) in obese mice but equal to or more efficacious in obese than lean mice at high doses (30–100 mg/kg). Leptin’s initial effects on weight loss could be explained by appetite suppression in both groups, but its effects on fat reduction were greater in leptin-treated than pair-fed mice, particularly in the lean group. Leptin also prevented the elevations in serum corticosterone and ketones found in pair-fed lean mice. These data allow a quantitative comparison of leptin sensitivity in obese vs. lean CD-1 mice and suggest that in mice where obesity is a function of outbreeding and age, leptin sensitivity is moderately reduced. Furthermore, although appetite suppression has a clear role in leptin’s effects on body weight, leptin may also have specific effects on lipid metabolism and mobilization that are different from the metabolic compensations that normally occur with food deprivation.

Leptin, the product of the obese gene, is synthesized in adipose tissue and can be detected in serum as a 16-kDa, α-helical protein (8, 20, 31). It was initially hypothesized that leptin acted as a signal of adiposity to the brain, which could, in turn, adjust adiposity by reducing caloric intake and/or increasing energy use (31). Consistent with the above hypothesis, leptin was shown to regulate body weight and appetite in the obese and hyperphagic ob/ob mouse, which, due to a recessive mutation, does not produce functional leptin (3, 11, 18, 24, 31). In addition, there was evidence that leptin bound to a hypothalamic receptor that had the capacity to influence other proteins through a signaling cascade typical of cytokine-like receptors (9, 16, 22, 28, 29). All of the above evidence led to the hope that leptin replacement would reduce adiposity in obese organisms.

The obese mutation is extremely rare in animals and humans, however. To date, it has only been observed in two hypoleptinemic, obese humans (23). Furthermore, in nonhuman animals, the obese mutation has only been observed in mice, and then on a limited number of backgrounds (2). In contrast, there is a large body of evidence showing that leptin levels are elevated in all other types of obesity and that body weight, percent body fat, and body mass index actually correlate in a positive manner with serum leptin or adipose mRNA for leptin (5, 7, 8, 12, 13, 19, 20, 25, 26).

The fact that most obese animals and humans have higher leptin levels than lean counterparts has led to the hypothesis that they may be resistant to their own leptin (5, 8). Consistent with a “leptin resistance” hypothesis is recent evidence showing that New Zealand obese (NZO), agouti (A), and high fat-fed AKR mice show either no response or respond only to higher doses of peripherally or centrally administered leptin (10, 30).

Resistance is a relative term, however; complete resistance is observed only in obese rats with leptin receptor mutations, of which there is no evidence in the above models. Exactly how resistant NZO, A, or fat-fed AKR mice are to endogenous leptin is unclear, because the studies described above did not test a wide range of doses in age- and strain-matched lean and obese mice. Without some index of sensitivity as a basis for comparison, it is difficult to discuss relative sensitivity to leptin in different models of obesity. A half-maximal effective dose derived from a wide range dose-response curve could serve as such an index. We have used this pharmacological index to compare leptin sensitivity in obese and lean mice given a wide range of doses (1–100 mg/kg) for a time period that was adequate to achieve asymptotic weight loss. We chose a murine model of obesity that is based on the age-related, natural body weight heterogeneity of an outbred strain of mice (CD-1).

CD-1 mice were originally bred from Swiss-ICR progenitors (Charles River). When these mice reach 10–12 mo of age, their body weights range from 29 to 62 g, with a mean weight of 42–47 g and an SD of 5–6.5, depending on age and housing conditions. We used these mice to answer the following questions about the obesity model and the effects of leptin on this model. 1) Does body weight correlate with leptin levels in these mice? 2) Does leptin have similar efficacy in mice at the heavy end of the distribution as it does in mice at the lean end of distribution? 3) Can leptin-induced changes in the body weight of obese and lean CD-1 mice be explained by its effects on food intake?

METHODS

Experiment 1

Initial characterization of a 10-mo-old population of female CD-1 mice was carried out on 83 female CD-1 mice that were
weighed and then bled from the retroorbital sinus under isoflurane anesthesia. These mice had been handled and group housed (5 or 6/cage) before measurement of final weights and blood collection. Mice were allowed food (Purina Rodent Chow; Newco, San Diego, CA) and water ad libitum. All animals were housed in the Amgen vivarium, where an ambient temperature of 21–23°C and a 12:12-h light-dark cycle (6:30 AM on, 6:30 PM off) were in effect.

Serum glucose, nonesterified free fatty acids (NEFA), cholesterol, β-hydroxybutyric acid (BHBA), and triglycerides were analyzed with the use of a Hitachi 717 blood chemistry analyzer, where glucose was measured using the hexokinase method (Boehringer Mannheim Biochemicals, Indianapolis, IN). Corticosterone was measured with the use of a competitive immunoassay system developed by Boehringer Mannheim Biochemicals. Insulin was measured with the use of a competitive immunoassay system with electrochemiluminescence on an Origin Analyzer (IGEN, Gaithersburg, MD). Leptin levels were measured with the use of a solid-phase sandwich enzyme immunoassay, with affinity purified polyclonal antibody immobilized in microtiter wells. Leptin level was calculated from standard curves generated for each assay with the use of recombinant mouse leptin. The detection limits of the assay are 70 pg/ml (25). All of the above variables were correlated with body weight with the use of simple regression analysis.

Experiment 2

A different population of 70 10-mo-old CD-1 mice was assigned to either lean or obese groups based on where their individual body weight fell under the weight distribution curve for this population. Body weights of these mice ranged from 36.6 to 62.2 g, with a mean weight of 46.6 g and an SD of 5.5. Mice that weighed $\leq 1.4$ SD from the mean were assigned to the lean group, and those that weighed $\leq 1.4$ SD from the mean were assigned to the obese group. Approximately 31% of the mice fell into either the lean or obese categories.

Obese and lean groups were further subdivided into seven dose/treatment groups: an uninjected group (UIJ), a PBS-treated group, and five leptin dose groups (1, 3, 10, 30, and 100 mg·kg$^{-1}$·day$^{-1}$). There were five mice/treatment group. Body weights were randomized as much as possible between treatment groups. All mice were group housed throughout the study (5/cage), with treatments randomized within each cage. Environmental conditions were identical to those described in experiment 1.

Obese and lean mice were given recombinant methionine murine leptin (r-Met-murine leptin) or the PBS vehicle by daily bolus intraperitoneal injection for a period of 33 days. Production and purification of r-Met-murine leptin, with the use of the Escherichia coli expression system, has been previously described (24). Injections were always given at the end of the dark cycle, and body weights were taken before the onset of the dark cycle every day. On the 12th day of treatment, blood was taken from the retroorbital sinus for measurement of serum leptin, lipids, and glucose. Mice were killed on day 34 (24 h after the last injection). At the time of death, mice were placed under isoflurane-induced anesthesia and blood was again taken from the retroorbital sinus. Carcasses were set aside for composition analysis.

Carcass composition was assessed using the methods of Leshner et al. (17). Water composition was determined by subtraction of carcass weight before and after a 5-day dehydration period. Fat was extracted with ethyl ether and ethyl alcohol from a preweighed portion of the ground, dried carcass, so that the percentage of fat could be calculated from the amount of material remaining after the extraction procedure. Lean mass was defined as the proportion of ground carcass that remained after dehydration and ether extraction.

Measurement of leptin and other serum chemistry variables were conducted as described in experiment 1.

Statistical analyses. Two-way ANOVAs were used to analyze the serum chemistry data, where the between-groups variables were body weight (obese vs. lean) and treatment (UIJ, PBS, or leptin dose). A mixed-design (group × treatment over time) repeated-measures ANOVA was used to analyze data gathered on the same mice over time, such as the body weight data. Fisher’s least significant difference (LSD) or least squares analysis was chosen for post hoc analysis in all cases. Half-maximal effective doses were estimated from a plot of percentage of maximal effect (y-axis) vs. log(10) dose.

Experiment 3

To compare leptin’s metabolic effects in obese vs. lean mice, we compared weight loss and serum chemistry changes in leptin-treated, PBS-treated, and pair-fed representatives of lean and heavy groups. Forty-eight 10- to 12-mo-old CD-1 mice were assigned to either lean or obese groups on the basis of where their individual body weight fell under the weight distribution curve for this population. Body weights of these mice ranged from 30.6 to 51.0 g, with a mean weight of 39.0 and an SD of 5.5. Mice that weighed $\leq 1.4$ SD from the mean were assigned to the lean group, and those that weighed $\geq 1.4$ SD from the mean were assigned to the obese group.

The obese and lean groups were further subdivided into three groups, a leptin-treated group, a vehicle-treated group, and a group that was pair-fed to the leptin-treated group. Body weights were randomized as much as possible between treatment groups. All mice were individually housed for at least 2 wk before characterizing the population by body weight and remained individually housed throughout the study. Furthermore, mice were allowed food (ground rodent chow) and water ad libitum, unless they were in the pair-feeding groups.

Obese and lean mice were given r-Met-murine leptin by continuous infusion for a period of 7 days with the use of a subcutaneously implanted Alzet mini-pump (1007D) that infused at a rate of 0.5 µl/h. All animals received $\sim 2.4$ mg·kg$^{-1}$·day$^{-1}$ of leptin. This route of administration maximized daily exposure to leptin (the half-life in rodents is only 30–40 min) (13), and the dose had previously been shown effective in lean mice (11). Vehicle controls were infused with PBS (pH 7.4). Pair-fed mice were given the same amount and type of food (ground rodent chow) as consumed by the individual leptin-treated counterparts the previous day. Body weight and food intake were monitored each day for all mice. On the 8th day after pump implantation (24 h after the last infusion day), blood was taken from the retroorbital sinus and mice were killed. Carcasses were weighed and dried for composition analysis. Carcass composition, serum chemistry, and leptin levels were measured as described in experiment 2.

Statistical analyses. Two-way ANOVAs were used to analyze the serum chemistry data, where the between-groups variables were body weight (obese vs. lean) and treatment (PBS, leptin, or pair-fed). A mixed design (group × treatment over time) repeated-measures ANOVA was used to analyze data gathered on the same mice over time, such as the food intake and body weight data. Fisher’s LSD or least squares analysis was chosen for post hoc analysis in all cases.
RESULTS

Experiment 1

The initial population had an average body weight of 43.4 g, with an SD of 6.3. Weights ranged from 29.7 to 55.7 g and formed a normal distribution. Serum leptin positively correlated with body weight ($r = 0.82; P < 0.0001$). The relationship between body weight and serum leptin is shown in Fig. 1. Serum NEFA vs. body weight was the only other serum variable that had a correlation coefficient even approaching the magnitude of the leptin-body weight relationship ($r = 0.50; P < 0.0001$).

Experiment 2

Mice in the obese group were significantly heavier (25%) than those in the lean group ($F(1,55) = 19.3; P < 0.0001$), with the average baseline weight for lean mice at 40.13 ± 0.28 g and the average weight for obese at 52.46 ± 0.36 g. Daily injection of r-Met-murine leptin (1–100 mg·kg$^{-1}$·day$^{-1}$) resulted in significant weight loss for both lean and obese groups of CD-1 mice after 33 days of treatment ($F(7,55) = 10.3; P < 0.0001$). This effect was both time ($F(32,1760) = 68.7; P < 0.0001$) and dose dependent ($Ps < 0.04–0.0001$). There was no significant interaction between group and treatment after 33 days of treatment. The complete dose-response function is expressed in Fig. 2, A and B. If average weight loss for obese and lean mice was collapsed over time during the period where asymptotic weight loss was achieved (days 24–33) and expressed as percent-age of maximal response vs. PBS [(loss at dose $x$ – loss at PBS)/(loss at highest dose – loss at PBS)·100] and then regressed against log dose, an index of half-maximal effective dose could be inferred for both groups. Calculated in the above manner, the half-maximal effective dose was ~0.5–0.7 log greater in the obese group (6–8 mg/kg for lean mice and 25–30 mg/kg for the obese mice). If percentage of maximal response was not corrected against the PBS group, the two groups had fairly similar half-maximal dose indexes (3 mg/kg for lean and 6–7 mg/kg for obese). Percentage of maximal response curves for body weight were also calculated with doses converted to total milligrams per mouse, in case dosing by body weight underestimated...
true effective doses for the obese group. The half-
maximal effective dose was still 0.5–0.7 log greater in
the obese group. The plots used to calculate these
indexes are shown in Fig. 3A (%maximal response with
the PBS correction), 3B (%maximal response without
the PBS correction), and 3C (%maximal response with
dose represented as total mg/mouse). Repeated-meas-
ures comparisons of weight loss between obese and
lean groups at individual doses did not reveal signifi-
cant differences, suggesting that rate of weight loss was
similar, regardless of preleptin weight. This can be
observed by comparing weight loss at individual doses
in Fig. 2, A and B.

Table 1 shows that at the time of death, body weights
were significantly lower in leptin-treated mice than in
PBS-treated counterparts [F(7,55) = 5.6; P < 0.0001]
whether they were obese or lean. The difference be-
 tween leptin-treated and PBS-treated groups reached
significance at the 10 mg/kg dose for the lean group
(P < 0.04) and at the 30 mg/kg dose for the heavy group
(P < 0.01).

Analysis of carcass composition data revealed that
fat was significantly reduced in both lean and obese
leptin-treated mice [F(7,124) = 17.39; P < 0.0001].
These differences became significant at the 1 mg/kg
dose for lean mice and at the 30 mg/kg dose for obese
mice (P values < 0.03–0.0001). Overall, the heavy
group of mice had two times more fat than their lean
counters (P < 0.0001). Lean mass was significantly
higher in the heavy group of mice [F(1,124) = 8.6; P <
0.004] and was altered after leptin treatment at the
3–100 mg/kg doses (P values < 0.01–0.0001) in the
same group. The dose for half-maximal fat loss (cal-
culated in the same manner as half-maximal weight loss,
corrected against PBS) was about three times lower in
lean mice (5 mg/kg for lean and 15 mg/kg for obese).
Again, if dose was represented as total milligrams per
mouse instead of milligrams per kilogram body weight,
the half-maximal effective dose was 0.5 log greater for
the obese group. The plot used to calculate half-
maximal fat loss is shown in Fig. 4A. Figure 4B
illustrates the same curve but with dose expressed as
total milligrams per mouse. Lean mass was not affected
by leptin treatment in the lean group. Carcass water
was significantly higher in obese mice [F(1,124) = 34.4;
P < 0.0001] but was not altered by leptin treatment in
either group. Carcass composition is summarized in
Table 1.

Leptin levels were significantly higher (2.4×) in the
heavy control groups than in the lean control groups
(P values < 0.01 vs. lean), as was serum NEFA (P < 0.03)
(see Table 2). After 33 days of treatment, even trough
leptin levels were elevated at the two highest doses
(P values < 0.001) in both groups. Two serum variables
were significantly reduced in a dose-dependent manner
after 33 days of leptin treatment: serum triglycerides in
lean mice [F(6,55) = 2.56; P < 0.03] and serum total
protein [F(6,55) = 6.8; P < 0.0001] in both groups at the
two highest doses (P values < 0.03–0.005). Serum
NEFA was elevated at the highest leptin dose in lean
mice (P < 0.0008) but not obese mice.

Fig. 3. Comparison of dose efficacy in lean vs. obese CD-1 mice for
body weight loss corrected against the PBS group with dose ex-
pressed as mg/kg (A), weight loss not corrected against the PBS group
(B), and weight loss corrected against the PBS group but with doses
expressed as total dose/mouse (C). For the body weight loss efficacy
index, average weight loss for obese and lean mice was collapsed over
time during the period where asymptotic weight loss was achieved
days 24–33). This variable was expressed as %maximal response vs.
PBS [(loss at dose x – loss at PBS)/(loss at highest dose – loss at
PBS)·100] and then regressed against log dose. Intersection of the
regression line at the 50% point with the x-axis provided an index of
half-maximal effective dose. B: average weight loss was expressed as
%maximal response vs. un.injected group (UIJ) rather than PBS.
Calculated in the manner shown for A, the half-maximal effective
dose was 6–8 mg/kg for lean mice and 25–30 mg/kg for obese
mice. Expression of dose as total/mouse resulted in half-maximal
effective doses for lean and obese mice that were similar to A.
Table 1. Effects of r-Met-murine leptin on carcass composition after 33 days of treatment in female CD-1 mice

<table>
<thead>
<tr>
<th></th>
<th>Baseline Wt, g</th>
<th>Final Wt, g</th>
<th>Water, g</th>
<th>Fat, g</th>
<th>Lean Mass, g</th>
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<td>Lean mice</td>
<td></td>
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<tr>
<td>Uninjected</td>
<td>39.9 ± 0.97</td>
<td>39.2 ± 1.2</td>
<td>22.2 ± 0.35</td>
<td>9.9 ± 0.8</td>
<td>7.1 ± 0.41</td>
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<tr>
<td>PBS</td>
<td>41.1 ± 1.1</td>
<td>40.5 ± 0.8</td>
<td>22.3 ± 0.7</td>
<td>11.5 ± 0.5</td>
<td>6.8 ± 0.22</td>
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<tr>
<td>r-Met-murine leptin</td>
<td></td>
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<td></td>
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<tr>
<td>1 mg/kg</td>
<td>40.6 ± 0.95</td>
<td>37.9 ± 0.69</td>
<td>23.5 ± 0.87</td>
<td>8.0 ± 1.3*</td>
<td>6.4 ± 0.31</td>
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<tr>
<td>3 mg/kg</td>
<td>40.9 ± 1.2</td>
<td>38.0 ± 1.8</td>
<td>22.5 ± 0.87</td>
<td>8.2 ± 1.1*</td>
<td>7.3 ± 0.19</td>
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<td>10 mg/kg</td>
<td>39.1 ± 1.3</td>
<td>35.7 ± 1.5*</td>
<td>22.4 ± 0.48</td>
<td>6.2 ± 0.94*</td>
<td>7.1 ± 0.42</td>
</tr>
<tr>
<td>30 mg/kg</td>
<td>39.9 ± 1.0</td>
<td>35.8 ± 1.4*</td>
<td>22.5 ± 0.25</td>
<td>5.3 ± 0.62*</td>
<td>7.9 ± 0.19</td>
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<td>100 mg/kg</td>
<td>39.1 ± 1.3</td>
<td>32.8 ± 1.3*</td>
<td>22.5 ± 0.73</td>
<td>2.9 ± 0.62*</td>
<td>7.4 ± 0.33</td>
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<td>Obese mice</td>
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<td></td>
</tr>
<tr>
<td>Uninjected</td>
<td>52.9 ± 3.1†</td>
<td>52.3 ± 2.6†</td>
<td>25.6 ± 0.61†</td>
<td>18.5 ± 1.9*</td>
<td>8.2 ± 0.51*</td>
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<tr>
<td>PBS</td>
<td>54.3 ± 1.2†</td>
<td>50.8 ± 0.85†</td>
<td>25.7 ± 1.5†</td>
<td>18.6 ± 0.92†</td>
<td>6.4 ± 0.5</td>
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<td>r-Met-murine leptin</td>
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<td></td>
</tr>
<tr>
<td>1 mg/kg</td>
<td>52.2 ± 1.6</td>
<td>50.1 ± 1.0</td>
<td>23.4 ± 0.44</td>
<td>19.8 ± 0.68</td>
<td>6.9 ± 0.44</td>
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<td>3 mg/kg</td>
<td>52.6 ± 1.2</td>
<td>49.9 ± 1.0</td>
<td>24.3 ± 0.93</td>
<td>16.7 ± 0.83</td>
<td>8.8 ± 0.28*</td>
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<tr>
<td>10 mg/kg</td>
<td>51.9 ± 1.5</td>
<td>49.2 ± 1.6</td>
<td>23.7 ± 1.4</td>
<td>17.6 ± 1.5</td>
<td>7.8 ± 0.49*</td>
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<tr>
<td>30 mg/kg</td>
<td>51.1 ± 2.3</td>
<td>45.2 ± 1.8*</td>
<td>25.8 ± 0.82</td>
<td>10.9 ± 0.85*</td>
<td>8.4 ± 0.55*</td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>51.7 ± 1.0</td>
<td>44.3 ± 2.4*</td>
<td>26.2 ± 0.46</td>
<td>10.2 ± 1.0*</td>
<td>7.9 ± 0.3*</td>
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</table>

Values are means ± SE. r-Met-murine leptin, recombinant methionine murine leptin. *P < 0.04–0.001 vs. PBS; †P < 0.03–0.01 vs. lean.

Experiment 3

Continuous infusion of r-Met-murine leptin (2.4 mg kg⁻¹·day⁻¹) for 7 days resulted in significant weight loss for both lean and obese groups of CD-1 mice. A repeated-measures ANOVA revealed a significant interaction among treatment, group, and time [F(12,108) = 4.6; P < 0.0001]. Analysis of this interaction revealed that leptin treatment reduced body weight in both obese (P < 0.047) and lean mice (P < 0.0004). Obese and lean mice that were pair-fed to leptin-treated counterparts also showed significant weight loss (Ps < 0.02–0.0001), with the magnitude of weight loss similar to their leptin-treated counterparts, as can be observed in Table 3.

Leptin also reduced food intake in both groups of mice. A repeated-measures ANOVA revealed significant treatment [F(1,27) = 15.3; P < 0.0006], group [F(1,27) = 6.0; P < 0.02], and time [F(6,162) = 17.0; P < 0.0001] main effects. Leptin treatment significantly reduced food intake in lean (P < 0.003) and obese (P < 0.03) groups, although the magnitude of this reduction was greater in lean mice (P < 0.02). Vehicle-treated obese mice did not eat significantly more than lean counterparts, as shown in Table 3.

Body fat was significantly reduced by leptin treatment in lean, but not obese, mice (see Table 3). Two-way ANOVA revealed significant treatment [F(2,39) = 3.6; P < 0.038] and group [F(1,39) = 124.4; P < 0.0001] effects. Although leptin reduced body fat in lean mice (P < 0.02), it did not significantly reduce body fat in pair-fed controls or in obese mice. There were, however, nonsignificant trends for body fat reduction in the pair-fed and obese mice. Leptin treatment did not significantly alter carcass water or lean mass in obese or lean mice.

Serum glucose, triglycerides, corticosterone, BHBA, and free fatty acids (FFA) were all significantly affected by leptin treatment and/or pair-feeding, as can be observed in Table 4. Glucose was significantly reduced in both leptin-treated and pair-fed lean mice [F(2,41) = 5.3; P < 0.009] (P values < 0.003, 0.03); it was not, however, altered by either manipulation in obese mice. Triglycerides were significantly reduced in both obese and lean leptin-treated and pair-fed mice [F(2,41) = 13.1; P < 0.0001] (P values < 0.0001–0.03). In obese mice, triglycerides were reduced more in pair-fed mice than in leptin-treated mice (P < 0.04), whereas the triglyceride reduction was equivalent in lean leptin-treated and pair-fed mice. Corticosterone was significantly affected only in lean, pair-fed mice, where it was increased (P < 0.02). BHBA was significantly reduced by leptin treatment in obese mice, but not in lean mice, as indicated by a significant group × treatment interaction [F(2,41) = 15.6; P < 0.0001]. Although BHBA was not affected by leptin treatment in lean mice, it was significantly increased in their pair-fed counterparts (P < 0.0001). A similar, but less dramatic, effect could be observed in the obese mice (P < 0.03). FFA were significantly elevated in the obese control group (P < 0.0001) and were reduced in both leptin-treated and pair-fed obese mice (P values < 0.0003–0.0004). Insulin was not significantly affected by treatment, but was significantly higher in obese mice, as reflected by a significant group effect [F(2,41) = 5.2; P < 0.03].

**DISCUSSION**

The CD-1 mouse can be portrayed as a model of moderate obesity in that the body weights of these outbred animals become heterogeneous with age, with the heaviest mice weighing 27–30 g more than the leanest mice. When obese vs. lean categories were compared, the average body weight of the obese group was 1.3 times greater than the average body weight of the lean group. The obese group also had twice the body fat and two to four times the leptin levels of their leaner counterparts. In addition, leptin levels correlated positively with body weight in these 10- to
12-mo-old mice, with a correlation coefficient similar to that reported for rodents and humans (5, 7, 8, 20, 25). The obese CD-1 mouse, then, is similar to other obese organisms in that they have significantly higher leptin levels (5, 8, 20, 25) than lean counterparts. Furthermore, their average baseline weight is in the same range (45–60 g) as some inbred models of obesity, such as the fat-fed AKR or C57Bl6J mouse (10, 30), the AY (2), and the NZO mouse (2).

The half-maximal effective dose of leptin for weight loss in obese CD-1 mice is between 25 and 30 mg/kg, which was ~0.5–0.7 log higher than that for lean mice, if it was corrected for weight loss due to vehicle alone. If the above index were not corrected for vehicle effects, there would be only a very small difference in half-maximal effective doses for obese vs. lean mice (3 mg/kg for lean vs. 6 mg/kg for obese), suggesting that injection stress was a more significant variable in obese mice than in lean.

The half-maximal effective dose for carcass fat was also shifted to the right in obese mice by one-half log. The shift in responsiveness was due to the fact that lean mice showed significant fat and weight loss at the lower dose range (3–10 mg/kg), which was not observed in obese counterparts. The overall magnitude of the shift was moderate, however, because obese mice lost just as much or more fat as lean mice at the high dose range (30–100 mg/kg).

Leptin-induced changes in weight and carcass fat were also accompanied by reductions in lean mass in the obese groups. Interestingly, even vehicle treatment appeared to reduce lean mass in the obese mice; again, this might suggest a heightened sensitivity to injection and handling stress in the obese group.

One could argue that the apparent lack of complete resistance in obese CD-1 mice is an artifact of our dosing regimen, in that we dosed animals in relationship to body weight, giving the obese mouse more leptin than lean counterparts. However, calculation of the half-maximal dose using total dose per mouse gives essentially the same result, in that obese CD-1 mice require a one-half log higher dose than lean counterparts to get the same reduction in body weight or fat. All of the above suggests that whereas obese CD-1 mice are less sensitive to the weight- and fat-reducing effects of leptin than their lean counterparts, they are not completely resistant to leptin; in fact, if the dose is high enough, they lose as much fat and body mass as lean counterparts.

There are reports in the literature suggesting that other nonmutant models of obesity, such as AKR or C57Bl6J mice fed a high-fat diet, might have similar sensitivity to leptin as the obese CD-1 mouse. Halasa et al. (10) showed that 25 mg/kg daily, given as two injections of 12.5 mg/kg, reduced body weight by ~10%, which is similar in magnitude to the weight change induced by 30 mg/kg in our obese CD-1 mice. Furthermore, van Heek et al. (30) demonstrated a significant reduction in food intake after one 10 mg/kg injection of leptin in fat-fed AKR mice and a nonsignificant trend for the same effect in C57Bl6J mice fed a 45% fat diet for 16 days (30). Interestingly, obese AY and NZO mice failed to respond at all to doses as high as 25 mg·kg<sup>-1</sup>·day<sup>-1</sup>, although they did show weight loss after central leptin administration, with the AY mouse being the most resistant to central leptin (10). All of these data might suggest that if lean vs. obese dose-effect curves were generated for each of these forms of obesity, one might observe that half-maximal effective doses would be shifted further to the right for mice with inbred mutations than for mice with either diet-induced obesity or obesity that is a function of age and outbreeding.
Table 2. Effects of r-Met-murine leptin given as a daily intraperitoneal bolus on serum chemistry after 33 days of treatment in female CD-1 mice

<table>
<thead>
<tr>
<th>Glucose (mg/dl)</th>
<th>Insulin (ng/ml)</th>
<th>Cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>NEFA (meq/l)</th>
<th>BHBA (meq/l)</th>
<th>Corticosterone (ng/ml)</th>
<th>Leptin (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lean mice</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uninjected</td>
<td>175.2 ± 12.9</td>
<td>3.80 ± 0.07</td>
<td>55.8 ± 2.5</td>
<td>171.4 ± 21.2</td>
<td>91.2 ± 16.9</td>
<td>2.0 ± 0.1</td>
<td>3.9 ± 0.29</td>
</tr>
<tr>
<td>PBS</td>
<td>186.2 ± 4.2</td>
<td>0.42 ± 0.15</td>
<td>66.2 ± 8.7</td>
<td>178.4 ± 13.9</td>
<td>75.2 ± 17.4</td>
<td>2.0 ± 0.1</td>
<td>3.78 ± 0.39</td>
</tr>
<tr>
<td>r-Met-murine leptin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 mg/kg</td>
<td>177.4 ± 11.2</td>
<td>0.18 ± 0.02</td>
<td>85.6 ± 8.7</td>
<td>152 ± 18.1</td>
<td>72.4 ± 16</td>
<td>2.1 ± 0.18</td>
<td>4.3 ± 0.36</td>
</tr>
<tr>
<td>3 mg/kg</td>
<td>197.2 ± 0.5</td>
<td>0.34 ± 0.09</td>
<td>83.8 ± 17.9</td>
<td>119.2 ± 14.7</td>
<td>51.6 ± 7.3</td>
<td>1.7 ± 0.17</td>
<td>3.7 ± 0.36</td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>201.2 ± 12.3</td>
<td>0.3 ± 0.1</td>
<td>75.7 ± 15.1</td>
<td>108.7 ± 17.3</td>
<td>56 ± 14.1</td>
<td>1.7 ± 0.25</td>
<td>3.3 ± 0.59</td>
</tr>
<tr>
<td>30 mg/kg</td>
<td>188 ± 5.4</td>
<td>0.84 ± 0.14</td>
<td>73.6 ± 6.2</td>
<td>165.2 ± 33.6</td>
<td>43.2 ± 7.5</td>
<td>2.6 ± 0.46</td>
<td>3.6 ± 0.47</td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>180.8 ± 9.3</td>
<td>0.58 ± 0.06</td>
<td>76.6 ± 10.6</td>
<td>126.2 ± 23.3</td>
<td>32 ± 3.9</td>
<td>3.4 ± 0.36</td>
<td>2.9 ± 0.59</td>
</tr>
</tbody>
</table>

Obese mice

<table>
<thead>
<tr>
<th>Glucose (mg/dl)</th>
<th>Insulin (ng/ml)</th>
<th>Cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>NEFA (meq/l)</th>
<th>BHBA (meq/l)</th>
<th>Corticosterone (ng/ml)</th>
<th>Leptin (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninjected</td>
<td>164.6 ± 9.4</td>
<td>0.74 ± 0.35</td>
<td>82.4 ± 14.6</td>
<td>218.8 ± 27</td>
<td>73.2 ± 8.9</td>
<td>3 ± 0.55</td>
<td>3.1 ± 0.46</td>
</tr>
<tr>
<td>PBS</td>
<td>203.4 ± 8.6</td>
<td>1.11 ± 0.56</td>
<td>79.2 ± 7.5</td>
<td>191.8 ± 21.8</td>
<td>71.2 ± 11.5</td>
<td>2.1 ± 0.21</td>
<td>3.6 ± 0.29</td>
</tr>
<tr>
<td>r-Met-murine leptin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 mg/kg</td>
<td>174.6 ± 12.2</td>
<td>0.44 ± 0.12</td>
<td>82.8 ± 12.8</td>
<td>119.2 ± 10.8</td>
<td>54.8 ± 12.5</td>
<td>1.7 ± 0.17</td>
<td>3.5 ± 0.29</td>
</tr>
<tr>
<td>3 mg/kg</td>
<td>167.6 ± 9.8</td>
<td>0.6 ± 0.3</td>
<td>91.8 ± 11.2</td>
<td>160.6 ± 29.5</td>
<td>58 ± 14.3</td>
<td>2.7 ± 0.54</td>
<td>3.8 ± 0.24</td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>208.4 ± 15.8</td>
<td>1.4 ± 0.53</td>
<td>78.4 ± 11.2</td>
<td>136 ± 27.5</td>
<td>56 ± 2.2</td>
<td>2.1 ± 0.18</td>
<td>4.7 ± 0.44</td>
</tr>
<tr>
<td>30 mg/kg</td>
<td>218.2 ± 4.1</td>
<td>0.56 ± 0.14</td>
<td>78.4 ± 11.2</td>
<td>181.8 ± 29.5</td>
<td>58.4 ± 7.5</td>
<td>2.4 ± 0.28</td>
<td>3.6 ± 0.49</td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>220.8 ± 15.7</td>
<td>1.4 ± 0.54</td>
<td>95.8 ± 6.3</td>
<td>200.4 ± 29</td>
<td>55.2 ± 7.9</td>
<td>2.7 ± 0.45</td>
<td>4.3 ± 0.41</td>
</tr>
</tbody>
</table>

Values are means ± SE. Blood was collected from fed mice 24 h after last leptin injection (at 3:00 PM). NEFA, nonesterified fatty acid; BHBA, β-hydroxybutyric acid.* P < 0.04–0.001 vs. PBS; † P < 0.03–0.01 vs. lean.

Leptin treatment reduced serum triglycerides in lean mice, as previously shown in ob/ob mice (18). Along with a reduction in triglycerides, this group of mice also showed a small elevation in serum FFA, which would fit a profile of increased lipid use. Although there was a trend for similar changes in obese mice, they did not reach statistical significance, which could also reflect a reduction in sensitivity to leptin. At the very high doses, serum total proteins were reduced in both groups; this, along with the marked elevation in trough leptin levels at 100 mg/kg, might suggest that long-term treatment with this dose might induce some changes in kidney clearance of leptin and other proteins. There are data indicating that leptin is cleared primarily through a renal mechanism (6).

When leptin was given as a continuous subcutaneous infusion at 2.4 mg/kg for 7 days, weight loss in obese mice was comparable to that observed for each group given the 10 mg/kg dose as a daily bolus for 33 days. After such a short treatment duration, weight loss in obese mice was already similar to that of lean mice given this regimen. This profile might suggest that 2.4 mg/kg given as a continuous infusion for 7 days is roughly equivalent to a dose between 10 and 30 mg/kg given as a daily bolus for 33 days. These data are consistent with other reports suggesting that leptin is more effective when given as a continuous infusion than as a daily bolus (10, 24).

Continuous infusion of leptin induced significant appetite suppression in both groups of mice. After 7 days of treatment (before appetite stabilization), lean mice had reduced their food intake more than obese mice (26 vs. 16%). Therefore, leptin-treated obese mice were still eating significantly more than their lean counterparts, which may be a component of the moderate reduction in leptin sensitivity seen in obese mice at low to moderate doses.

Pair-fed mice from both groups were also eating significantly less than vehicle controls, which resulted in weight loss that was similar to leptin-treated counterparts for obese and lean groups. Despite this, pair-fed lean mice only lost one-half of the fat of leptin-treated mice, as previously observed (11, 18). These data generalize the findings of Levin et al. (18) with an outbred strain of mice, supporting the idea that leptin does not reduce body weight by appetite suppression alone, at least in lean mice. Because a similar trend existed for obese mice, it is possible that this phenomena would also have been observed in obese mice if a higher dose had been used or the study had been carried out for a longer period.

After 7 days of infusion, leptin-induced changes in serum triglycerides, FFA, and glucose could all be attributed to effects on food consumption, because none

Table 3. Effects of r-Met-murine leptin on food intake, body weight, and carcass fat in obese versus lean CD-1 female mice

<table>
<thead>
<tr>
<th>Food Intake</th>
<th>Weight Loss</th>
<th>Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obese vehicle</td>
<td>4.8 ± 0.39</td>
<td>1.70 ± 0.49</td>
</tr>
<tr>
<td>Obese leptin treated</td>
<td>4.01 ± 0.37</td>
<td>3.62 ± 0.66</td>
</tr>
<tr>
<td>Obese pair-fed</td>
<td>NA</td>
<td>3.3 ± 0.94</td>
</tr>
<tr>
<td>Lean vehicle</td>
<td>4.47 ± 0.26</td>
<td>0.25 ± 0.39</td>
</tr>
<tr>
<td>Lean leptin treated</td>
<td>3.22 ± 0.3</td>
<td>4.3 ± 0.5</td>
</tr>
<tr>
<td>Lean pair-fed</td>
<td>NA</td>
<td>4.75 ± 0.56</td>
</tr>
</tbody>
</table>

Values are means ± SE in g (n = 7 or 8 mice/group). Leptin was administered by continuous infusion into the subcutaneous tissue at an approximate dose of 2.4 mg-kg⁻¹-day⁻¹ for a period of 7 days. Food intake is expressed as average g eaten on day 7, weight loss is expressed as average loss from baseline weight on day 7, and fat is expressed in g. Baseline weights were as follows: obese, PBS = 45.1 ± 1.3; obese, leptin = 44.9 ± 1.2; obese, pair-fed = 44.1 ± 1.0; lean, PBS = 35 ± 0.7; lean, leptin = 34.5 ± 1.1; lean, pair-fed = 34.5 ± 0.8. * P < 0.04–0.0001 vs. obese vehicle treated; † P < 0.02–0.006 vs. lean vehicle treated.
of these variables were significantly different when leptin-treated and pair-fed controls were compared. In obese mice, leptin and food restriction reduced serum triglycerides and FFA but did not affect glucose, whereas in lean mice, leptin and food restriction reduced serum triglycerides and glucose but did not affect FFA. Because neither obese nor lean mice had reached a stabilized weight loss after 7 days, the differences between obese and lean profiles probably reflect the different stages of fat loss and reduced food intake for the two groups.

BHBA and corticosterone were elevated in pair-fed lean mice, which is consistent with the idea that lean pair-fed mice were demonstrating a metabolic profile similar to that observed in severe diet restriction. (Pair-fed mice were eating roughly 26% less than vehicle-treated mice.) Leptin-treated mice, however, did not show these changes in corticosterone or BHBA. These findings are consistent with a recent report stating that serum BHBA was not altered in rats made hyperleptinemic by adenovirus gene transfer but was elevated in pair-fed controls (27). The dissociation between leptin treatment and food restriction suggests that leptin treatment could have altered the hormonal and metabolic phenomena normally associated with food restriction, as recently suggested by Ahima et al. (1).

Leptin might act to alter the metabolic phenomena associated with food restriction by inducing enzymes that mediate FFA oxidation. Shimabukuro et al. (27) demonstrated that leptin treatment increased intracellular FFA oxidation and reduced intracellular triglyceride formation. In contrast, tissue from pair-fed animals only showed reduced triglyceride formation (27, 32). Furthermore, leptin, but not pair-feeding, promoted the induction of enzymes that mediate FFA oxidation as well as uncoupling protein-2, an enzyme that can induce thermogenesis in white fat (32). All of this data could suggest that leptin somehow promotes a more complete oxidation of FFA than does food restriction or starvation, perhaps by increasing the rate of intracellular FFA oxidation and using the energy produced from FFA oxidation for thermogenesis, as suggested by Unger and colleagues (27, 32). Furthermore, it is possible that if leptin acted directly to promote FFA oxidation in liver, pancreas, and adipose tissue, the necessity for corticosterone-mediated fuel mobilization would be reduced. Thus leptin’s effects on FFA oxidation might provide an explanation for the finding that leptin-treated animals do not show enhanced corticosterone release.

Alternatively, leptin could act directly on the hypothalamic-pituitary-adrenal (HPA) axis to prevent the rise in corticosterone that normally occurs during energy deprivation. Long-term leptin receptors have been localized most reliably in areas of the hypothalamus known to mediate hormonal/metabolic responses (9, 16, 22, 28, 29). Furthermore, Ahima et al. (1) showed that peripheral leptin administration prevented the elevation in both ACTH and corticosterone normally observed in starved mice. In addition, Heiman et al. (13) demonstrated that leptin prevented the normal elevation in corticotropin-releasing hormone release induced by hypoglycemia in rat hypothalamic explants. Furthermore, the HPA axis of mice and rats with leptin ligand or receptor defects is overresponsive to stressors (2), suggesting that leptin might be a developmental requirement for a normal HPA axis response to stress.

If leptin acted as a general inhibitor of the HPA axis response to stressors such as starvation, corticosterone-induced fuel mobilization would be inhibited during periods of energy deprivation. Without the glucocorticoid response, intracellular FFA oxidation might be the only mechanism available that could provide sufficient usable fuel to each tissue. Whether or not a blunted glucocorticoid response to starvation would actually promote an increase in intracellular FFA oxidation is unclear but testable.

Perspectives

Our data address two important issues concerning leptin: 1) leptin can induce equivalent weight loss in obese, outbred mice and lean counterparts if the dose range is increased by 0.5–0.7 log for the obese group and 2) leptin appears to reduce adiposity in both lean and obese mice through a mechanism that is very different from that observed in starvation. Although outbred 10- to 12-mo-old obese CD-1 mice show moderately reduced sensitivity to leptin (0.5–0.7 log), their response to leptin at high doses (30–100 mg·kg⁻¹·day⁻¹) is of equal magnitude to those of lean counterparts. This outcome should not be particularly surprising in view of two facts: 1) these mice do not have any known leptin receptor mutations and 2) these mice already have much higher endogenous leptin levels, yet are still
obese, suggesting that they are at least somewhat insensitive to their own leptin. Interestingly, inbred mutation models of murine obesity appear to be less sensitive to leptin than obese CD-1 mice, even though the mutations that define these models do not involve the leptin receptor. Thus obese CD-1 mice and inbred mutation models of obesity might be added to the continuum already suggested by several investigators to describe leptin responsivity: ob/ob >> lean mice >> obese CD-1 = diet-induced obese mice >> inbred mutation models > receptor mutants. Exactly why inbred mutation models, such as AY or NZO, appear to be so insensitive to leptin is unclear but the fact that they do suggests that these mutations target systems important to leptin receptor function.

Whether our CD-1 mice were lean or obese, leptin’s effects on adipose mass, corticosterone, or ketone levels were very different from those observed in food-restricted animals. Leptin did not alter corticosterone or ketones, whereas animals that were eating the same amount of food (pair-fed controls) showed dramatic elevations in both measures, which is the typical response found during starvation. How does leptin dissociate the impact of its effects on appetite from its effects on fat metabolism? Unger and colleagues (27, 32) have shown that leptin regulates the expression of enzymes involved in intracellular fatty acid oxidation as well as uncoupling protein-2 in a manner that is very different from that observed in starvation. These effects of leptin result in an increased rate of intracellular FFA oxidation and thermogenesis, which is not observed in pair-fed controls.

Because leptin also appears to prevent the starvation-induced elevation in corticosterone (which induces fatty acid mobilization during starvation), we have speculated that leptin might promote a combination of events that could radically increase the efficiency of fat metabolism over that found in food deprivation. Leptin would not only double the rate of intracellular FFA oxidation, but also reduce FFA mobilization into blood, thereby not only double the rate of intracellular FFA oxidation, and thermogenesis, which is not observed in pair-fed controls.

The above hypothesis suggests that exogenous leptin alters the normal hormonal/metabolic response to the state of energy deprivation that leptin produces. We could infer that the mechanism for a normal response to starvation must be intact for leptin to use fat so efficiently. Therefore, it may be important to consider the HPA axis and metabolic status of each obesity model before forming conclusions about leptin’s effects in that particular model.

We are indebted to Jason Moore and Margery Nicolson for analysis of serum leptin levels; to Larry Ross and Silvia Copon for serum chemistry analysis; and to Randy Hecht, Dwight Winters, and Tom Boone for manufacture and purification of murine leptin.

Address for reprint requests: M. A. Pelleymenter, Neurocrine Biosciences, 3050 Science Park Rd., San Diego, CA 92121-1102.

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