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A Leptin Dose-Response Study in Obese (*ob/ob*) and Lean (+/?) Mice

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ABSTRACT

This experiment determined the amount of leptin required to correct different abnormalities in leptin-deficient *ob/ob* mice. Baseline food intakes and body weights of lean (+/?) and obese (*ob/ob*) C57Bl/6J <ob> mice were recorded for 7 days. An Alzet miniosmotic pump was placed in the peritoneal cavity of each mouse and delivered 0, 1, 2, 5, 10, or 42 μg/day human leptin for 7 days. In *ob/ob* mice, 2 μg leptin/day reduced food intake and body weight, and increased hypothalamic and brain stem serotonin concentrations. All fat pads were reduced 35–40% by 10 μg leptin/day, and liver weight, lipid, and glycogen decreased. Serum insulin and glucose were reduced in all leptin-treated *ob/ob* mice, and levels were normalized by 10 μg/day leptin. Low rectal temperatures of *ob/ob* mice were corrected by 10 and 42 μg/day leptin. These doses also increased brown adipose tissue uncoupling protein expression. The only responses in lean mice were a transient reduction in food intake and weight loss with 10 or 42 μg/day leptin. This study shows enhanced leptin sensitivity in *ob/ob* mice and suggests that increased temperature and sympathetic activity are indirect responses to high concentrations of protein. (*Endocrinology* **139:** 8–19, 1998)

GENETICALLY obese *ob/ob* mice have a single gene mutation that results in a syndrome of obesity that includes diabetes, infertility, hypothyroidism, hypercorticoidism, low sympathetic activity, and impaired thermoregulation (1). Parabiosis studies with *ob/ob* mice indicated that the mutation causes a deficiency in a circulating lipostatic factor (2). In 1994, Zhang *et al.* (3) identified the product of the gene mutation in *ob/ob* mice that was responsible for their obesity and was also the presumed circulating factor. This protein, leptin, has the structure of a long chain helical cytokine (4) and is expressed in adipose tissue in proportion to adipocyte size (5, 6).

Many investigators have established that administration of leptin to genetically obese *ob/ob* mice reduces food intake and body weight (7–10). The suppression of food intake is mediated by a hypothalamic splice variant of the leptin receptor, OB-Rb, which has a long intracellular domain (11). OB-Rb is mutated in genetically obese *db/db* mice (12), and as expected, they are unresponsive to the hypophagic effects of both peripheral and central administration of leptin (7–10). Obese humans have high circulating concentrations of leptin, but are not responsive to its effects on food intake (13). As there is a concentration gradient between serum and cerebrospinal fluid leptin (14, 15), it has been hypothesized that a rate-limited transport system prevents peripheral leptin from activating the central receptor that suppresses food intake. The limitation on transport may be due either to binding proteins in the circulation (16) or to a specific transport protein (17). It has been proposed that one of the leptin receptor subtypes, with a short intracellular domain, may function as a transport protein and regulate leptin uptake into the brain (12).

In addition to suppressing food intake, peripheral administration of leptin to *ob/ob* mice corrects infertility (18), reverses hyperglycemia and hyperinsulinemia (9), and increases body temperature and metabolic rate (9). In lean mice, leptin has minimal effects on food intake, but causes the loss of body fat, presumably due to a leptin-induced increase in energy expenditure (19). Leptin has been shown to increase norepinephrine (NE) turnover in brown, but not white, adipose tissue, suggesting that the metabolic effect of leptin is attributable to activation of the sympathetic nervous system (20). This would be consistent with leptin-deficient *ob/ob* mice having low sympathetic tone compared with lean controls (1).

Leptin has a circulating half-life of approximately 30 min, is released in a pulsatile manner from adipose tissue, and demonstrates a circadian rhythm in circulating levels with a nocturnal elevation in concentration (13, 21). The majority of studies investigating the effects of leptin on physiological parameters have administered the protein in one or two daily injections in doses ranging from 3–250 μg/day (7–10). We have found that this method of protein administration causes excessive, intermittent elevations of the serum leptin concentration (22). In this experiment recombinant human leptin was infused for 7 days at doses ranging from 0–42 μg/day from Alzet miniosmotic pumps placed in the peritoneal cavity of the mice. This method of administration provided constant delivery of protein (0–1.75 μg/h), but did not mimic the diurnal changes in leptin release. The objectives of the study were 2-fold. The first was to determine which of the physiological responses to leptin in *ob/ob* mice occurred with low doses of constantly infused leptin and which were in-

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duced only with large doses of protein. This would allow separation of physiological from potentially pharmacological responses. The second objective was to determine which of the responses observed in *ob/ob* mice were also apparent in lean mice that already have normal circulating concentrations of leptin and do not respond to the satiety effects of peripherally administered protein.

Materials and Methods

Five-week-old female C57B1/6J lep <ob>, *ob/ob*, and lean (+/?) mice were purchased from Jackson Laboratory (Bar Harbor, ME) and housed individually with continuous free access to chow (Purina mouse chow 5015, Ralston Purina, St. Louis, MO) and water. Room temperature was maintained at 26 C, and lights were on for 12 h/day from 0600 h. Body weights and food intakes were recorded daily at 0700 h, and rectal temperatures of mice were measured four times during the study at 0900 h, twice before and twice after pump placement, using a thermistor probe (Thermistor thermometer model 8110–20, Cole Palmer Instrument Co., Chicago, IL).

After 4 days of baseline measurements of body weight and food intake, lean and *ob/ob* mice were divided into six weight-matched groups, and an Alzet pump (model 1007D, Alza Corp., Palo Alto, CA) was placed in the peritoneal cavity of each mouse. The pumps delivered 0, 1, 2, 5, 10, or 42 μg human recombinant leptin/day in a total volume of 12 μl, and PBS was used as a diluent. Leptin was a gift from Zymo-Genetics Corp. (Seattle, WA). After pump placement, measurements of daily food intake and body weight were continued, and rectal temperatures were measured after 2 and 4 days of infusion.

All mice were decapitated in the morning of day 7 of leptin infusion. Trunk blood was collected for serum analysis of insulin (Rat RIA kit, Linco Research, St. Louis, MO), corticosterone (RIA kit, ICN Radiochemical, Irvine, CA), glucose (Sigma Diagnostic Kit 510. Sigma Chemical Co., St. Louis, MO), and human leptin (Human Leptin RIA kit, Linco Research). The carcass, liver, pancreas, ovaries, uterus, spleen, adrenals, heart, kidney, and inguinal, perirenal, retroperitoneal, mesenteric, gonadal, and intrascapular brown fat were dissected and weighed. Tissues smaller than 250 mg were weighed on a microbalance (Cahn C-31 microbalance, Cahn Instruments, Cerritos, CA). The liver, hypothalamus, brown fat, and gonadal fat were snap-frozen. All procedures were approved by the Pennington Biomedical Research Center Institutional Animal Care and Use Committee.

TRIzol reagent (Life Technologies, Grand Island, NY) was used to extract total RNA from gonadal fat for measurement of leptin expression by Northern blot analysis, as described previously (6), and from brown fat for measurement of uncoupling protein (UCP) messenger RNA (mRNA) by Northern blot analysis as described previously (23), using a complementary DNA probe generously provided by Dr. Daniel Riquier. The hypothalamus, brain stem, and cortex were dissected and snap-frozen in liquid nitrogen for analysis of monoamines by HPLC, as described previously (24). Liver tissue was frozen for subsequent analysis of glycogen by the method of Lo *et al.* (25) and for lipid content by chloroform-methanol extraction. The triceps surae muscle from mice in the 0, 2, and 10 μg leptin/day groups was frozen for enzyme analysis. A 5% homogenate was made of the muscle samples in Tris buffer (175 mM KCl, 2 mM EDTA, and 10 mM Tris-HCl, pH 7.4). Citrate synthase was used as a general marker for oxidative capacity and was assayed as described by Srere (26). β-Hydroxyacyl coenzyme A dehydrogenase (HOAD) activity was used as a marker for fatty acid oxidation potential and was determined as described by Askew *et al.* (27). Hexokinase activity was used a marker for glucose utilization potential and was assayed according to the method of Uyeda and Racker (28).

Small pieces of frozen liver from lean and *ob/ob* mice treated with either 0 or 10 μg/day leptin were homogenized in Krebs bicarbonate buffer, pH 7.5, containing protease inhibitors (10 μM leupeptin, 2 U/ml aprotinin, and 1 μM phenylmethylsulfonylfluoride). A crude membrane fraction was prepared by centrifuging the homogenate for 10 min at 3,000 $\times$ *g* and then recentrifuging the supernatant at 11,000 $\times$ *g* for 20 min. Samples (40 μg) of both the resulting supernatant and pellet were separated by SDS-PAGE in a 9% acrylamide gel in Tris glycine buffer (25 mM Tris, 192 mM glycine, and 0.1% SDS, pH 8.3). The proteins were transferred to a polyvinylidene difluoride membrane (Boehringer Mannheim, Mannheim, Germany) in 25 mM Tris, 192 mM glycine, and 20% methanol. Leptin receptor was detected by Western blot using a polyclonal rabbit antimouse OB-R antibody, a gift from Affinity BioReagents (Golden, CO). The blot was developed using a chemiluminescence system (BM Chemiluminescence Blotting Substrate, Boehringer Mannheim) according to the manufacturer's directions using the first antibody at a 1:4,000 dilution and an antirabbit IgG POD second antibody (Boehringer Mannheim).

Statistical analysis

For each genotype, the response variables food intake and body weight were separately modeled as a repeated measures ANOVA over the course of the experiment. To provide overall tests of the dosage effect of leptin on each variable, a profile analysis was effected by testing the appropriate contrasts corresponding to the parallel, coincident, and level profiles hypotheses for days 4–7 of infusion after the mice had recovered from the surgery. Comparisons of the effects of different leptin dosages on the response measures on specific days of infusion were obtained by testing the appropriate contrasts corresponding the hypothesis of interest; where appropriate, *P* values reported for these contrasts have been adjusted for multiple comparisons by Bonferroni's method. Where possible, tests were conducted using Satterthwaite's approximation to determine the appropriate degrees of freedom (29).

Rectal temperatures were analyzed by repeated measures ANOVA, with day as the repeated measure. Organ weights, leptin mRNA, UCP mRNA, liver glycogen, liver lipid, muscle enzymes, serum measurements, and brain neurotransmitter concentrations were analyzed by two-way ANOVA to determine whether there were genotype effects and by one-way ANOVA with *post-hoc* Duncan's multiple range test to determine treatment effects within each genotype. The one-way ANOVA was performed even when the two-way ANOVA did not show an interaction between genotype and leptin, as the value for some of the parameters, such as body fat and serum hormones, were so much greater in obese than in lean mice that variance within *ob/ob* groups masked substantial treatment effects in lean animals. The SAS System for Windows (release 6.12, SAS Institute, Cary, NC) was used for computations.

Results

Food intakes of *ob/ob* and lean mice are shown in Fig. 1. The dramatic reduction in food intake on the first day of leptin infusion was partially due to surgery. During subsequent days of infusion, 1 μg leptin/day tended to reduce food intake compared with that of control obese mice, but repeated measures analysis showed that the difference was significant only on days 1 and 5 of infusion (adjusted $P < 0.003$ and $P < 0.03$, respectively). All other leptin doses (2, 5, 10, and 42 μg/day) suppressed food intake in the obese mice from the first day of infusion, and intake remained below that of controls until the end of the experiment, although the intake of mice receiving 2 and 5 μg/day gradually increased during the 7 days of infusion. A maximal effect was reached with 10 μg leptin/day, at which dose food intake remained at approximately 20% of the control intake. In lean mice, food intake was transiently suppressed on the first 2 days of leptin infusion in mice receiving 10 or 42 μg leptin/day. Lower doses of leptin had no significant effect on food intake. Daily body weights of the mice are shown in Fig. 2. *ob/ob* mice receiving 0 or 1 μg/day leptin gained weight during the 7 days of infusion, and there was no significant difference between the body weights of the two groups. The body weights of mice receiving 2 or 5 μg/day plateaued at a lower level than their preinfusion weight, whereas mice receiving 10 or 42 μg/day constantly lost weight. Lean mice receiving 42 μg leptin/day weighed significantly less than controls

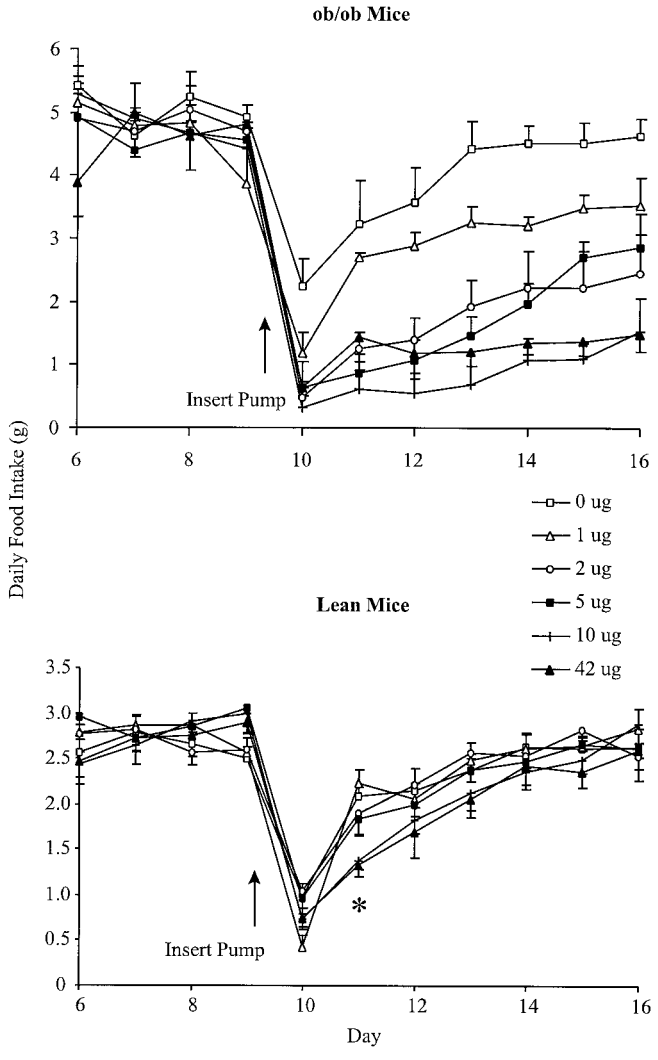

FIG. 1. Daily food intakes of lean and obese mice. Data are the mean ± SEM for groups of six mice. An Alzet pump delivering 0, 1, 2, 5, 10, or 42 μg/day human recombinant leptin was placed in the peritoneal cavities of the mice on the day indicated by the *arrow*.

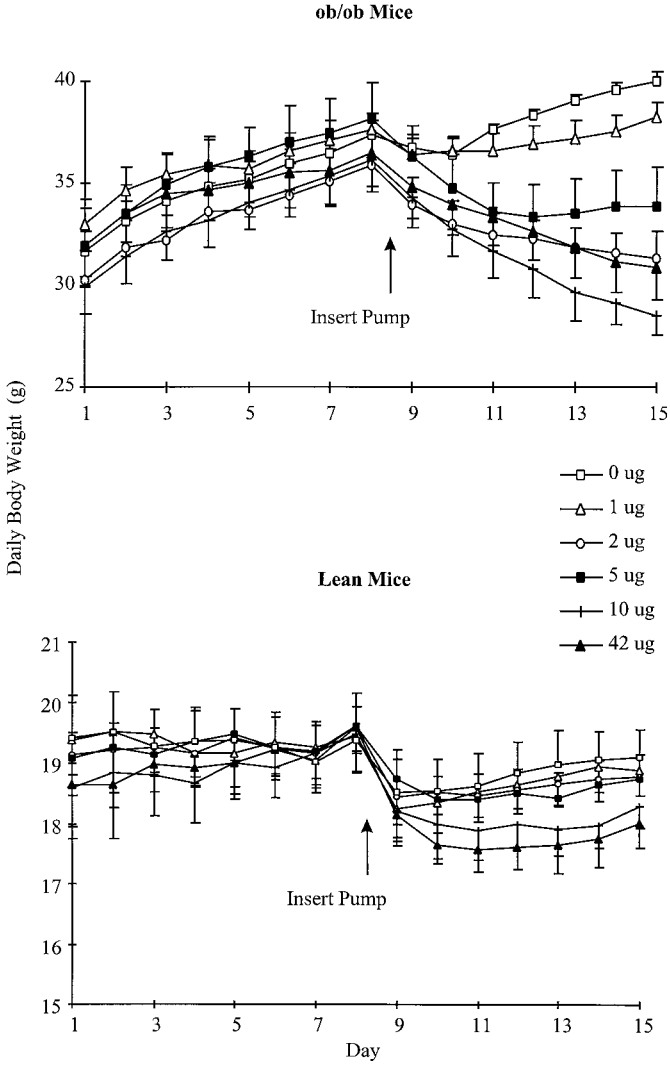

FIG. 2. Daily body weights of lean and obese mice. Data are the mean ± SEM for groups of six mice. An Alzet pump delivering 0, 1, 2, 5, 10, or 42 μg/day human recombinant leptin was placed in the peritoneal cavities of the mice on the day indicated by the *arrow*. Statistical analysis revealed significant differences in body weights of *ob/ob* mice receiving 2 μg leptin/day, or more, compared with those of controls. In lean mice, the animals receiving 10 or 42 μg/day leptin weighed significantly less than controls.

from day 2 of infusion. For mice receiving 10 μg/day leptin, the weight difference was significant from day 3. The 5 μg/day group weighed significantly less ($P < 0.02$) than controls on days 4, 5, and 6 of leptin infusion, but not on day 7, and mice receiving 2 μg/day weighed significantly less than controls on days 5 and 6 of infusion.

Rectal temperatures of the mice are shown in Fig. 3. Repeated measurements of temperature caused a progressive increase in temperature of all animals. Repeated measures two-way ANOVA showed a significant effect of genotype ($P < 0.00001$) and no significant effect of leptin dose, but a significant effect of day ($P < 0.0001$) on temperatures of the mice. There were also significant interactions between genotype and day ($P < 0.0001$) and among genotype, leptin dose, and day ($P < 0.05$). Before leptin infusion, the temperatures of all obese mice were significantly lower than those of lean animals. By the second and fourth days of infusion, the temperatures of *ob/ob* mice receiving 10 or 42 μg/day leptin were no longer different from those of lean animals. There was no effect of leptin on the temperatures of lean mice. Results from measurements of UCP mRNA expression are shown in Fig. 4. Two-way ANOVA showed no significant effect of genotype or leptin on UCP expression. However, when a one-way ANOVA was performed within the obese genotype, UCP expression was significantly elevated in *ob/ob* mice receiving 10 and 42 μg leptin/day compared with that in other obese mice.

The weights of different adipose depots are shown in Table 1. In obese mice, all fat pad weights were reduced with a leptin dose of 2 μg/day or more. A maximal effect was obtained with 10 μg/day. In the lean mice, the only significant change in fat pad weight was the retroperitoneal fat of mice in the 10 μg/day group. The other fat pads also tended to be reduced at leptin doses of 10 and 42 μg/day, but

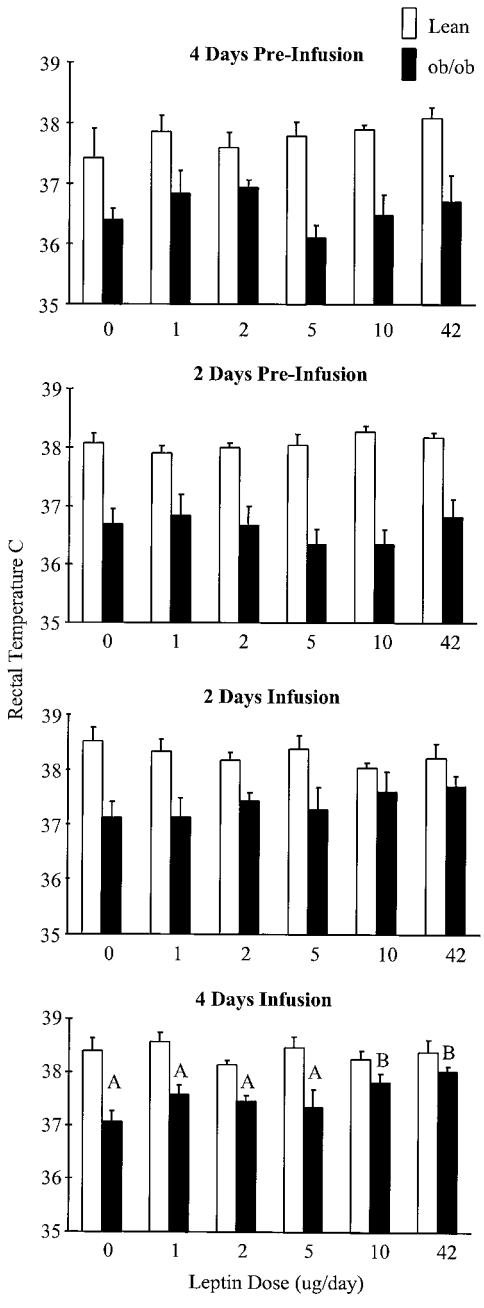

FIG. 3. Rectal temperatures of lean and obese mice measured twice before leptin infusion and twice during leptin infusion. Data are the mean ± SEM for groups of six mice. Obese mice had significantly lower temperatures than lean mice, except in the 10 and 42 μg/day groups on the second and fourth days of leptin infusion. *Superscripts* indicate a significant difference between treatment groups within the obese genotype on the fourth day of infusion. There were no significant differences within lean or obese genotypes on any other day.

differences did not reach statistical significance. Gonadal fat leptin mRNA expression is shown in Fig. 5. Two-way ANOVA showed a significant effect of both genotype ($P < 0.0001$) and leptin dose ($P < 0.0004$) and a significant interaction between the two variables ($P < 0.0013$). *Post-hoc* Duncan's multiple range test demonstrated that leptin expression was substantially higher in adipose tissue from *ob/ob* mice

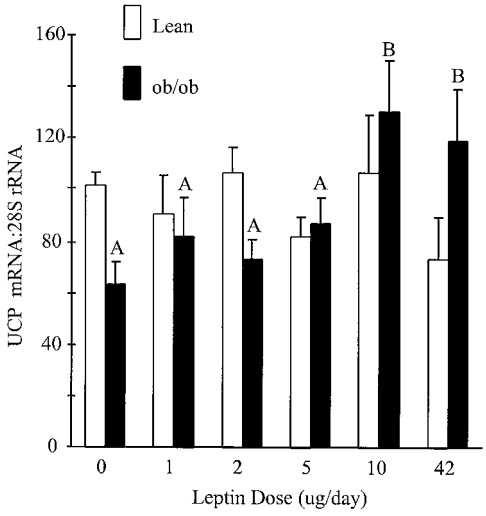

FIG. 4. Expression of UCP mRNA intrascapular brown fat from lean and obese mice infused for 7 days with increasing amounts of human recombinant leptin. Data are the mean ± SEM for groups of four to six mice. *Superscripts* indicate significant differences in expression between groups of obese mice. There was no effect of leptin on UCP expression in lean mice.

than in that from lean mice. There was no effect of leptin administration on leptin expression in lean mice, but the two highest doses of leptin caused a significant reduction in expression in *ob/ob* mice.

Organ weights are shown in Table 2. Two-way ANOVA showed genotype effects on the weights of kidneys, adrenals, and ovaries. There was no statistically significant effect of any dose of leptin on the weight of any of the organs measured in either obese or lean mice. Liver composition is shown in Table 3. Livers of *ob/ob* mice were significantly larger than those in lean mice and contained substantially more lipid and glycogen. In contrast to the other organs, leptin significantly reduced liver weight in obese mice starting at the 1 μg/day dose and reaching a maximal effect with 10 μg/day. Liver lipid was reduced at all doses of leptin, and liver glycogen content was decreased by 2 μg/day. None of the doses of leptin had any effect on liver weight, liver lipid, or liver glycogen content in lean mice. A Western blot of the short form leptin receptors is shown in Fig. 6. There was no detectable long form (OB-Rb) receptor, and there was no obvious effect of leptin on the amount of short form receptor present in livers of lean or *ob/ob* mice, although the results suggest that leptin receptors were expressed at higher levels per unit protein in livers of lean mice than in those of obese mice.

Triceps surae muscle hexokinase, citrate synthase, and HOAD activity are shown in Table 4. Enzyme activity was only measured in tissue from three treatment groups per genotype. HOAD activity was significantly higher in *ob/ob* than in lean mice, but genotype had no effect on citrate synthase or hexokinase activity. There was no effect of leptin on enzyme activity in *ob/ob* mice, but both hexokinase and HOAD activities were increased in lean mice treated with 2 μg leptin/day. This increase was reversed by 10 μg leptin/day.

The results of serum analysis are shown in Table 5. In both

TABLE 1. The effect of leptin infusion on the weight of fat depots in lean and *ob/ob* mice

Leptin dose (μg/day)	Rp (mg)	Inguinal (mg)	Perirenal (mg)	Mesenteric (mg)	Gonadal (mg)	Brown (mg)	Total (g)
ob/ob mice							
0	785 ± 45^{a}	3790 ± 154^{a}	477 ± 46^{a}	1141 ± 41^{a}	4058 ± 160^{a}	291 ± 24^{a}	10.3 ± 0.2^{a}
1	$665 \pm 46^{a,b}$	3786 ± 210^{a}	553 ± 75^{a}	$1042 \pm 22^{a,b}$	$3487 \pm 60^{a,b}$	$240 \pm 63^{a,b}$	$9.8 \pm 0.2^{a,b}$
2	$544 \pm 51^{b,c}$	$3175 \pm 210^{a,b}$	291 ± 28^{b}	$775 \pm 68^{c,d}$	$2907 \pm 120^{b,c,d}$	$174 \pm 24^{b,c}$	7.7 ± 0.4^{c}
5	$581 \pm 77^{b,c}$	$3191 \pm 365^{a,b}$	312 ± 37^{b}	$909 \pm 73^{b,c}$	$3198 \pm 310^{b,c}$	$188 \pm 16^{b,c}$	$8.4 \pm 0.8^{b,c}$
10	440 ± 36^{c}	2390 ± 180^{c}	313 ± 54^{b}	653 ± 57^{d}	2261 ± 230^{d}	118 ± 14^{c}	6.1 ± 0.5^{d}
42	489 ± 86^{c}	$2828 \pm 193^{b,c}$	269 ± 52^{b}	712 ± 64^{d}	$2543 \pm 340^{c,d}$	122 ± 10^{c}	$6.8 \pm 0.6^{c,d}$
Lean mice							
0	84 ± 7.9^{a}	564 ± 43	66 ± 6.8	155 ± 29	454 ± 41	83 ± 7	1.4 ± 0.1
1	80 ± 17^{a}	500 ± 79	64 ± 7.5	109 ± 15	446 ± 71	89 ± 4	1.3 ± 0.2
2	64 ± 11^{a}	482 ± 73	64 ± 10.6	98 ± 12	452 ± 71	95 ± 10	1.3 ± 0.2
5	74 ± 6.5^{a}	533 ± 40	59 ± 4.9	110 ± 11	466 ± 38	98 ± 11	1.3 ± 0.1
10	36 ± 8.5^{b}	312 ± 66	49 ± 7.3	87 ± 10	258 ± 50	73 ± 7	0.8 ± 0.1
42	$54 \pm 10^{a,b}$	397 ± 69	52 ± 9.2	102 ± 10	325 ± 63	83 ± 12	1.0 ± 0.2
Two-way ANOVA: *P* (F,df)							
Genotype	0.0001 (1101,1)	0.0001 (1033,1)	0.0001 (260,1)	0.0001 (1170,1)	NS	0.0001 (70,1)	0.0001 (1010,1)
Leptin	0.0001 (9.0,5)	0.0001 (9.4,5)	0.0001 (6.8,5)	0.0001 (16,5)	NS	0.0002 (5.8,5)	0.001 (15,5)
Interaction	0.0004 (5.3,5)	0.0006 (5.1,5)	0.0002 (5.8,5)	0.0001 (10,5)	NS	0.0006 (5.2,5)	0.001 (10,5)

Data are the mean ± SEM for groups of six mice infused with PBS or leptin for 7 days. The statistical summary represents a two-way ANOVA, demonstrating genotype effects. Statistically significant differences within genotype were determined by one-way ANOVA and subsequent Duncan's multiple range test; different *superscript letters* indicate significant differences between treatment groups within a genotype. Rp, Retroperitoneal. Total is the sum of the weights of the five dissected fat pads.

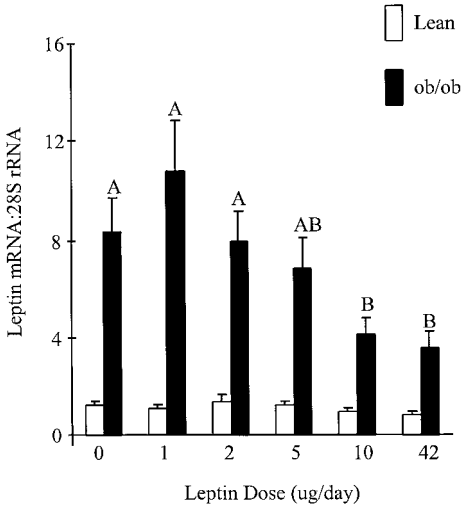

FIG. 5. Expression of leptin mRNA measured in gonadal fat from lean and obese mice after 7 days of infusion with increasing amounts of leptin. Data are the mean ± SEM for groups of four to six mice. Leptin expression was measured by Northern blot analysis and expressed as a ratio to 28S ribosomal RNA. *Superscripts* indicate significant differences in levels of expression in fat from obese mice. There was no effect of leptin on expression in tissue from lean mice.

lean and obese mice, serum leptin tended to increase with increased levels of infusion. A statistically significant difference was detected between controls and animals given 10 or 42 μg/day. There was no difference in leptin concentrations measured in mice infused with 10 or 42 μg/day leptin, suggesting that the pumps were unable to deliver the highest concentrations of protein efficiently. There was a significant genotype effect on serum insulin, glucose, and corticosterone, all of which were elevated in *ob/ob* mice compared with lean mice. Hyperinsulinemia in obese mice was improved significantly with leptin doses as low as 2 μg/day, and with 10 μg leptin/day, the serum insulin level was similar to that in lean mice. There was no change in the serum insulin concentration in any group of lean animals, and serum glucose was significantly reduced only by 42 μg leptin/day. There was a trend for increasing doses of leptin to suppress serum corticosterone in obese mice, but it did not reach statistical significance ($P < 0.09$). In lean mice, there was no effect of leptin on corticosterone ($P < 0.8$).

The results of HPLC analysis of monoamines and their metabolites in brain stem, hypothalamus, and frontal cortex are shown in Tables 6 and 7. Table 6 shows concentrations of serotonin (5-HT), its metabolite 5-HIAA, and the ratio of the two as an index of 5-HT metabolism. Two-way ANOVA indicated a significant genotype effect on 5-HT and 5-hydroxyinoleacetic acid (5-HIAA) concentrations in the hypothalamus and brain stem, with both compounds being present at higher concentrations in obese than in lean animals. 5-HT metabolism was different between lean and obese mice only in the brain stem. There were no effects of genotype on concentrations in the frontal cortex. Within genotypes, leptin administration caused significant elevations in 5-HT metabolism in obese, but not lean, mice. Leptin doses of 10 or 42 μg/day caused significant increases in 5-HIAA concentrations, compared with control values, in both the brain stem and hypothalamus of *ob/ob* mice. Leptin had no effect on the concentrations of any other neurotransmitter measured in the three brain areas dissected. Therefore, Table 7 summarizes the effect of genotype on the concentrations of monoamines within the three brain regions. In the brain stem, there was a significant genotype effect on the concentrations of all monoamines and metabolites measured, except for 3-methoxy, 4-hydroxyphenylethylene glycol (MHPG), MHPG/NE, and homovanillic acid (HVA)/dopamine (DA). In the hypothalamus there was no significant genotype effect on 3,4-dihydroxyphenylacetic acid (DOPAC) or DA concentrations or on the DOPAC/DA ratio; however, there were significant differences in the MHPG, MHPG/NE, and HVA/DA contents of tissue from lean and *ob/ob* mice. In the frontal cortex, there was a genotype effect on HVA, DOPAC/

TABLE 2. Organ weights of lean and obese mice treated with leptin

Leptin dose (μg/day)	Heart (mg)	Kidney (mg)	Spleen (mg)	Pancreas (mg)	Ovaries (mg)	Uterus (mg)	Adrenals (mg)
ob/ob mice							
0	108 ± 3	260 ± 14	48.4 ± 2.7	214 ± 11	7.3 ± 0.5	13.8 ± 0.8	7.8 ± 0.5
1	99 ± 2	266 ± 6	49.5 ± 2.6	219 ± 14	7.7 ± 1.5	15.6 ± 1.6	6.7 ± 0.7
2	95 ± 6	233 ± 9	63.4 ± 7.8	187 ± 7	6.6 ± 0.5	17.6 ± 1.7	6.1 ± 0.6
5	104 ± 5	264 ± 15	54.3 ± 1.3	199 ± 8	7.3 ± 1.1	15.7 ± 1.5	7.0 ± 0.7
10	91 ± 4	240 ± 9	89.3 ± 36	165 ± 28	6.3 ± 1.4	20.6 ± 2.7	7.0 ± 0.6
42	95 ± 3	238 ± 9	58.0 ± 6.5	152 ± 28	6.7 ± 1.2	23.9 ± 6.6	6.0 ± 0.4
Lean mice							
0	94 ± 2	217 ± 4	57.7 ± 3.3	180 ± 6	8.5 ± 1.1	27.6 ± 5.8	6.0 ± 0.2
1	99 ± 6	208 ± 7	57.9 ± 4.8	189 ± 5	10.2 ± 1.0	34.1 ± 6.1	6.4 ± 0.6
2	94 ± 3	211 ± 7	57.5 ± 5.3	182 ± 5	9.7 ± 1.1	57.8 ± 24.8	5.7 ± 0.3
5	89 ± 3	209 ± 6	56.7 ± 3.7	180 ± 13	8.9 ± 0.7	29.2 ± 3.1	5.7 ± 0.2
10	94 ± 4	210 ± 6	61.7 ± 3.7	174 ± 12	9.8 ± 0.9	52.0 ± 13.2	5.5 ± 0.5
42	91 ± 4	201 ± 4	54.4 ± 3.4	154 ± 13	9.8 ± 1.3	45.6 ± 20.6	5.3 ± 0.5
				Two-wayANOVA: *P*(F,df)			
Genotype		0.0001 (73,1)	NS	NS	0.007 (16.3,1)		0.04 (4.4,1)
Leptin		0.07 (2.2,5)	NS	0.01 (3.4,5)	NS		NS
Interaction		NS	NS	NS	NS		NS

Data are the mean ± SEM for six mice infused with PBS or leptin for 7 days. The statistical summary represents a two-way ANOVA, testing for a genotype effect. There were no significant differences between treatment groups within a genotype for any organ, analyzed by one-way ANOVA.

TABLE 3. Liver composition of lean and *ob/ob* mice treated with leptin

Leptin dose (μg/day)	Liver wt (g)	Liver lipid (mg)	Liver glycogen (mg)
ob/ob mice			
0	2.27 ± 0.13^{a}	364.2 ± 46.3^{a}	5.8 ± 0.8^{a}
1	1.99 ± 0.09^{b}	193.7 ± 32.2^{b}	$5.1 \pm 0.8^{a,b}$
2	1.48 ± 0.07^{c}	165.2 ± 35.5^{b}	3.0 ± 0.2^{c}
5	1.67 ± 0.12^{c}	113.0 ± 21.1^{b}	$3.8 \pm 0.9^{b,c}$
10	1.19 ± 0.06^{d}	158.1 ± 29.2^{b}	$3.7 \pm 0.5^{b,c}$
42	1.18 ± 0.09^{d}	109.8 ± 10.6^{b}	3.1 ± 0.2^{c}
Lean mice			
0	0.83 ± 0.02	56.1 ± 3.8	1.8 ± 0.2
1	0.83 ± 0.04	59.7 ± 4.3	1.7 ± 0.1
2	0.80 ± 0.01	53.0 ± 4.4	2.0 ± 0.3
5	0.83 ± 0.04	64.7 ± 6.5	1.8 ± 0.3
10	0.84 ± 0.02	50.1 ± 3.3	1.7 ± 0.2
42	0.78 ± 0.04	53.6 ± 3.7	1.6 ± 0.3
Two-way ANOVA: *P* (F,df)			
Genotype	0.0001 (219,1)	0.001 (104,1)	0.001 (89,1)
Leptin	0.0001 (8.2,5)	0.0001 (7.4,5)	0.04 (2.5,5)
Interaction	0.0001 (7.3,5)	0.0001 (7.5,5)	0.04 (2.6,5)

Data are the mean ± SEM for six mice infused with PBS or leptin for 7 days. The statistical summary represents a two-way ANOVA, testing for genotype effects. Different *superscript letters* indicate significant differences between treatment groups within a genotype, determined by one-way ANOVA and subsequent Duncan's multiple range test.

DA, and HVA/DA concentrations. MHPG was below detectable levels in this tissue.

Discussion

In this experiment, lean and genetically obese *ob/ob* mice were infused with increasing doses of recombinant human leptin for 7 days to define genotypic differences in response and to determine which responses to leptin were observed at low concentrations of protein and which required large amounts of protein. Analysis of serum human leptin by RIA indicated that we achieved our goal of causing progressive increases in circulating leptin concentrations in both lean and obese mice up to the 10 μg/day dose. As similar circulating concentrations were found in animals infused with 10 and 42 μg/day leptin, it is possible that leptin precipitated from the highly concentrated solution used to fill the 42-μg pumps or that some of the protein bound to the internal walls of the pump. Alternatively, mice may have a mechanism for clearing excessive amounts of protein from the circulation. Detection of leptin in serum from *ob/ob* mice treated with PBS indicated that the RIA was not specific for leptin, but it did permit comparison of relative concentrations of protein in serum from the different treatment groups. The lowest infusion dose of 1 μg/day did not increase detectable leptin beyond that measured in mice receiving PBS.

All doses of leptin greater than 1 μg/day caused significant reductions in the food intake and body weight of *ob/ob* mice. Low doses of leptin (2 and 5 μg/day = 0.08 and 0.2 μg/h) caused an initial drop in food intake that was partially reversed by the end of the experiment and resulted in stabilization at a reduced body weight. In contrast, the two highest doses of leptin caused a stable reduction in food

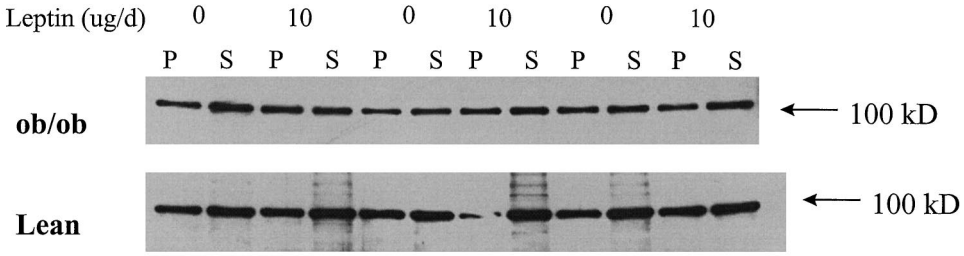

FIG. 6. Liver short form leptin receptor detected by Western blot using a polyclonal antibody raised to the extracellular membrane portion of the receptor. The *top blot* is liver from *ob/ob* mice, and the *bottom blot* is liver from lean mice infused with either 0 or 10 μg/day leptin. Tissue was homogenized and centrifuged at 3,000 × *g* for 10 min The supernatant was centrifuged at 11,000 × *g* for 20 min. Both the pellet (P) and the supernatant(s) were analyzed for receptor. Forty micrograms of protein were loaded in each lane. The 100-kDa band represents the receptors with a short intracellular domain (OB-Ra, OB-Rc, OB-Rd, and OB-Rf). There was no effect of leptin treatment on receptor in lean or obese mice.

TABLE 4. Muscle enzyme activity in lean and *ob/ob* mice infused with leptin for 7 days

Leptin dose (μg/day)	Hexokinase	Citrate synthase	HOAD
ob/ob mice			
0	1.38 ± 0.36	20.8 ± 3.2	2.54 ± 0.33
2	2.03 ± 0.09	31.3 ± 0.2	3.14 ± 0.14
10	1.40 ± 0.08	24.5 ± 4.5	2.57 ± 0.25
Lean mice			
0	1.76 ± 0.01[a]	26.9 ± 1.3	2.09 ± 0.0[a]
2	1.97 ± 0.06[b]	28.8 ± 0.7	2.44 ± 0.11[b]
10	1.54 ± 0.0[c]	27.3 ± 0.5	1.88 ± 0.05[a]
Two-way ANOVA: *P* (F,df)			
Genotype	NS	NS	0.002 (11.9,1)
Leptin	0.01 (5.2,2)	0.07 (96,2)	0.09 (2.6,2)
Interaction	NS	NS	NS

Data are the mean ± SEM for groups of four to six mice. Enzyme activity was measured in the triceps muscles collected from animals that had been infused with 0, 2, or 10 μg/day recombinant human leptin. The statistical summary represents a two-way ANOVA used to detect genotype effects. Different *superscript letters* indicate statistically significant differences between treatment groups within a genotype, determined by one-way ANOVA and *post-hoc* Duncan's multiple range test.

intake and continuous weight loss in obese mice. Brown adipose UCP levels and rectal temperature were also elevated with high doses of leptin, as discussed below, and it is likely that the associated elevation of energy expenditure contributed to weight loss in these mice. In lean mice, only the two highest doses of leptin caused significant changes in food intake and body weight. These results demonstrate that *ob/ob* mice have an increased sensitivity to the energy balance effects of leptin compared with that in lean mice. Although the intake of lean mice returned to control levels, there was no evidence of compensatory hyperphagia, suggesting that when the mice became resistant to the feeding effects of leptin, they were also insensitive to the existing reduction in body weight.

The ability of lean mice to develop resistance to the satiety aspects of leptin suggest that these animals are a more appropriate model than *ob/ob* mice for investigating leptin activity in humans. Obese humans and mice made obese by dietary means have elevated circulating concentrations of leptin but maintain a normal food intake (30–32). Central infusion of leptin into dietary obese mice inhibits intake (32), indicating that the resistance to peripheral leptin is caused by a failure to transport the protein to the hypothalamic long form, OB-Rb, receptor, which is responsible for the hypophagic effects of leptin (12). A similar rate-limiting transport system appears to be present in humans, as a concentration gradient in leptin is maintained between blood and cerebrospinal fluid (14, 15). The adaptive mechanism that limits leptin transport to the brain may be due to circulating binding proteins that limit the amount of free protein available for transport (16) or to a rate-controlled transport system at the blood-brain barrier (14). In *ob/ob* mice, this adaptive mechanism is either absent or substantially inhibited. The gradual increase in food intake of *ob/ob* mice given 2 or 5 μg/day leptin suggest that they have a limited ability to prevent leptin from reaching central receptors that control food intake.

Weight loss in *ob/ob* mice was accompanied by a reduction in body fat content, and there was no obvious site-specific response. In obese mice, increasing doses of leptin caused progressive loss of hepatic lipid and glycogen stores; however, by the end of this experiment, liver lipid and glycogen levels in mice given the two highest doses of leptin were still twice those found in lean mice. Despite the substantial change in liver composition and metabolism, there was no change in the level of expression of hepatic short form leptin receptors (OB-Ra, OB-Rc, OB-Rd, and OB-Rf), which have been shown to have signaling capability (33, 34). As reported previously (35), the long form of the leptin receptor, OB-Rb, was not detectable in the liver. If the change in liver composition had been directly mediated by leptin, a change in receptor number may have been expected as liver energy stores declined. The design of this experiment did not allow us to determine which responses in the mice were direct effects of leptin and which were secondary to the state of negative energy balance induced by leptin.

In lean mice, all fat pads were also reduced by 30–50% in animals treated with 10 μg/day leptin compared with those in controls; however, the difference was not statistically significant due to the relatively small size of the pads even in control animals. This observation confirms the hypothesis of Levin *et al.* (19) that leptin has metabolic effects, independent of those associated with hypophagia, that lead to a loss of body fat in both lean and *ob/ob* mice. As leptin had no significant effect on the hepatic lipid or glycogen content of lean mice, these results also suggest that the metabolic effect is tissue specific, diverting nutrients from adipose tissue to other tissues that have a higher metabolic rate. This concept

TABLE 5. The effect of leptin infusion on serum hormone and glucose levels in lean and obese mice

Leptin dose (μg/day)	Human leptin (ng/ml)	Insulin (ng/ml)	Glucose (mmol/L)	Corticosterone (ng/ml)
ob/ob mice				
0	1.5 ± 0.1^{a}	13.6 ± 3.3^{a}	25 ± 3^{a}	76 ± 23
1	1.6 ± 0.1^{a}	10.8 ± 2.5^{a}	$20 \pm 1.1^{a,b}$	40 ± 21
2	$3.7 \pm 1.4^{a,b}$	4.6 ± 1.4^{b}	18 ± 3.1^{b}	24 ± 2
5	$3.6 \pm 0.7^{a,b}$	2.6 ± 1.0^{b}	16 ± 1.1^{b}	21 ± 6
10	8.0 ± 2.5^{c}	1.4 ± 0.6^{b}	14 ± 1.4^{b}	28 ± 7
42	$6.4 \pm 1.3^{b,c}$	2.0 ± 0.6^{b}	15 ± 0.4^{b}	22 ± 6
Lean mice				
0	1.6 ± 0.3^{a}	1.1 ± 0.1	16 ± 1.0^{a}	23 ± 7
1	2.1 ± 0.3^{a}	1.1 ± 0.2	16 ± 0.6^{a}	20 ± 3
2	$3.0 \pm 0.5^{a,b}$	1.0 ± 0.3	16 ± 0.5^{a}	24 ± 4
5	$3.8 \pm 1.1^{a,b}$	1.0 ± 0.2	$15 \pm 1.1^{a,b}$	16 ± 3
10	6.8 ± 1.2^{c}	0.8 ± 0.4	16.1 ± 0.9^{a}	29 ± 12
42	$5.0 \pm 1.2^{b,c}$	0.8 ± 0.2	13.0 ± 0.5^{b}	17 ± 4
Two-way ANOVA: *P* (F,df)				
Genotype	NS	0.0001 (43,1)	0.003 (10.1,1)	0.04 (4.6,1)
Leptin	0.001 (8.8,5)	0.0001 (7.8,5)	0.0008 (4.9,5)	0.04 (2.6,5)
Interaction	NS	0.0001 (7.5,5)	0.03 (2.8,5)	NS

Data are the mean ± SEM for six mice infused with PBS or leptin for 7 days. The statistical summary represents a two-way ANOVA, testing for genotype effects. Different *superscript letters* indicate significant differences within a genotype, determined by one-way ANOVA and subsequent Duncan's multiple range test.

was supported by the measurement of muscle enzyme activity. In *ob/ob* mice, there was no significant effect of leptin treatment on any of the three enzymes measured, suggesting that glucose and fatty acid metabolism in these tissues was not substantially changed, even with 10 μg/day leptin. In lean mice, hexokinase and HOAD activities increased with a low dose of leptin and then decreased with the higher dose. These changes were small, and significance was due to the absence of variance in some of the treatment groups, but they may be representative of a shift in nutrient utilization from glucose to fatty acid oxidation, consistent with fatty acids being mobilized from adipose tissue.

Measurements of serum insulin and glucose showed that 2 μg/day human leptin caused a significant reduction in basal serum insulin and glucose levels in *ob/ob* mice, confirming previous reports that leptin improves glucose clearance in *ob/ob* mice (9, 10). The improvement cannot be entirely attributed to the reduction in food intake and body fat content of the mice, as Pellymounter *et al.* (9) found a reduction in serum insulin with a dose of leptin that did not change the body weights of *ob/ob* mice. Emillson *et al.* (36) have shown that leptin directly inhibits glucose-stimulated insulin release from pancreatic β-cells; however, the changes in *ob/ob* mice indicate an improvement in tissue insulin responsiveness, leading to a reduced requirement for insulin, rather than an inhibition of insulin release in the absence of a change in glucose uptake. If this had been the case, leptin-treated mice would have had lower insulin, but higher serum glucose, concentrations than controls. *ob/ob* mice given 10 μg/day leptin had serum insulin and glucose concentrations equivalent to those in lean controls. In these animals, which had a significantly reduced food intake and were mobilizing body fat, it is likely that a combination of factors, including direct effects of leptin in tissue insulin sensitivity and a reduced glucose load, contributed to the drop in serum insulin and glucose concentrations.

In vitro studies have shown that leptin induces insulin resistance in HepG2 cells (37), a hepatocellular carcinoma cell line, and rat adipocytes (38). These observations are not consistent with improved insulin responsiveness in leptin-treated *ob/ob* mice. There are a number of possible explanations for the discrepancy. The first is that we measured basal insulin and glucose levels when the animals were in a nonfed state. It is possible that leptin changes glucose-stimulated insulin release and insulin responsiveness, which would not have been detected in this experiment. Another explanation is that *ob/ob* mice are abnormal in their response to leptin. This would not be too surprising, as *ob/ob* mice have an increased sensitivity to leptin and are the only animals that remain hypophagic in response to peripherally administered leptin (7, 9, 32, 39). In addition, there was no effect of leptin on basal serum insulin concentrations in lean mice, and non-insulin-dependent diabetes is associated with both elevated leptin and insulin levels (40, 41). Finally, *in vitro* studies may not be representative of *in vivo* responses due to the absence of appropriate feedback systems, compensatory mechanisms, and secondary responses to leptin treatment. Studies of *in vivo* glucose utilization in leptin-treated animals are required to confirm the relevance of *in vitro* studies to the whole animal response to leptin. In addition to reducing serum insulin and glucose, 2 μg/day leptin appeared to reverse the hypercorticoidism of *ob/ob* mice, although the 68% drop in the average corticosterone concentration was not statistically significant. This change in circulating corticosterone may also have contributed to the improved insulin status of *ob/ob* mice, as glucocorticoids inhibit glucose uptake (42).

Others have speculated that the metabolic effects of leptin are associated with activation of the sympathetic nervous system (20). Sympathetic tone is reduced in *ob/ob* mice that are leptin deficient (1), and leptin treatment has been reported to increase norepinephrine turnover in brown, but not white, adipose tissue (20). In this experiment, brown adipose tissue UCP expression was used as an indirect index of sympathetic activity and was increased only in *ob/ob* mice infused with 10 or 42 μg/day leptin. These results demonstrate that

TABLE 6. Serotonin and 5-HIAA (nanograms per mg tissue) in brain stem, hypothalamus, and cortex of lean and *ob/ob* mice treated with leptin

Leptin dose (μg/day)	Brain stem			Hypothalamus			Frontal cortex		
	5-HT	5-HIAA	5-HIAA/5-HT	5-HT	5-HIAA	5-HIAA/5-HT	5-HT	5-HIAA	5-HIAA/5-HT
ob/ob mice									
0	1.29 ± 0.03	0.74 ± 0.04^{a}	0.57 ± 0.02^{a}	2.66 ± 0.08	1.00 ± 0.02^{a}	0.38 ± 0.01^{a}	0.79 ± 0.13	0.70 ± 0.08	0.93 ± 0
1	1.36 ± 0.05	0.88 ± 0.07^{a,b}	0.64 ± 0.03^{a,b}	2.70 ± 0.09	1.23 ± 0.05^{a,b}	0.46 ± 0.02^{a,b}	0.78 ± 0.07	0.68 ± 0.04	0.90 ± 0
2	1.45 ± 0.10	1.05 ± 0.06^{b,c}	0.74 ± 0.06^{a,b,c}	2.83 ± 0.15	1.71 ± 0.35^{b}	0.59 ± 0.09^{b}	0.69 ± 0.05	0.61 ± 0.05	0.90 ± 0
5	1.25 ± 0.14	0.93 ± 0.12^{a,b,c}	0.74 ± 0.02^{a,b,c}	2.65 ± 0.14	1.42 ± 0.12^{a,b}	0.54 ± 0.05^{a,b}	0.88 ± 0.14	0.65 ± 0.06	0.80 ± 0
10	1.52 ± 0.05	1.34 ± 0.16^{d}	0.89 ± 0.11^{c}	2.99 ± 0.09	1.73 ± 0.21^{b}	0.58 ± 0.08^{b}	0.84 ± 0.09	0.80 ± 0.06	0.99 ± 0
42	1.44 ± 0.06	1.20 ± 0.07^{c,d}	0.84 ± 0.06^{b,c}	2.96 ± 0.09	1.62 ± 0.08^{b}	0.55 ± 0.03^{b}	0.76 ± 0.08	0.72 ± 0.08	0.97 ± 0
Lean mice									
0	1.24 ± 0.08	0.80 ± 0.04	0.65 ± 0.04	2.49 ± 0.14	1.18 ± 0.03	0.48 ± 0.03	0.90 ± 0.13	0.69 ± 0.06	0.80 ± 0
1	1.213 ± 0.05	0.77 ± 0.06	0.64 ± 0.05	2.52 ± 0.09	1.21 ± 0.06	0.48 ± 0.03	0.81 ± 0.06	0.80 ± 0.03	1.99 ± 0.
2	1.26 ± 0.07	0.89 ± 0.14	0.70 ± 0.08	2.69 ± 0.11	1.34 ± 0.15	0.50 ± 0.05	1.64 ± 1.10	0.82 ± 0.25	1.20 ± 0
5	1.29 ± 0.06	0.84 ± 0.04	0.65 ± 0.03	2.84 ± 0.14	1.27 ± 0.09	0.45 ± 0.03	0.85 ± 0.12	0.90 ± 0.06	1.08 ± 0
10	1.22 ± 0.09	0.82 ± 0.04	0.68 ± 0.04	2.76 ± 0.21	1.31 ± 0.07	0.49 ± 0.04	0.71 ± 0.04	0.82 ± 0.26	1.20 ± 0
42	1.21 ± 0.06	0.80 ± 0.04	0.66 ± 0.02	2.59 ± 0.15	1.31 ± 0.05	0.52 ± 0.04	0.74 ± 0.04	0.80 ± 0.07	1.09 ± 0
Two-way ANOVA: *P* (F,df)									
Genotype	0.0004 (14,1)	0.0001 (20,1)	0.03 (5.0,1)	0.03 (4.9,1)	0.02 (5.4,1)	NS	NS	NS	NS
Leptin	NS	0.003 (4.1,5)	0.03 (2.7,5)	NS	0.006 (3.7,5)	0.08 (2.1,5)	NS	0.07 (3.27,1)	0.08 (3.
Interaction	NS	0.005 (3.8,5)	0.08 (2.1,5)	NS	NS	NS	NS	NS	NS

Data are the mean ± SEM for six mice infused with PBS or leptin for 7 days. The statistical summary represents a two-way ANOVA, testing for effects of genotype and leptin dose. Different *superscript letters* indicate statistically significant differences between treatment groups within a genotype, determined by one-way ANOVA and subsequent Duncan's multiple range test.

much higher doses of leptin are required to increase UCP expression and sympathetic activity than are required to change food intake and cause weight loss in either lean or *ob/ob* mice. Changes in rectal temperatures of *ob/ob* mice paralleled the increase in UCP expression. It is well established that thermoregulation is impaired in both *ob/ob* mice, which are deficient in leptin, and *db/db* mice, which have a mutated long form leptin receptor (1). As the temperatures of *ob/ob* mice receiving 10 or 42 μg/day leptin were not different from those of lean mice, it appears that high doses of leptin mediate either an increase in heat production or a reduction in heat loss. Both of these responses would be consistent with increased sympathetic activity, its associated activation of brown adipose tissue, and vasoconstriction in the skin (43). However, it is well established that cytokines cause a febrile response (44), although this is usually a transient effect in conditions of trauma or infection (45), and leptin has been shown to stimulate macrophage cytokine production and phagocytic activity *in vitro* (46). Therefore, it is possible that the effect on body temperature was also partially due to a leptin-induced increase in concentrations of inflammatory cytokines. We did not measure any cytokines other than leptin in this study due to a limitation in the amount of serum available.

Others (18) have reported that 14 days of leptin injections caused a significant increase in ovarian and uterine weights in *ob/ob* mice, but in this experiment we did not find any significant effect of leptin on the weights of reproductive organs in either lean or *ob/ob* mice. The uterine weights in *ob/ob* mice tended to increase with leptin treatment, but the difference did not reach statistical significance due to the large variability within each group. There was no indication of leptin having any effect on the ovaries of *ob/ob* mice, which were significantly smaller than those of lean animals. The difference in these results and those reported previously (18) may be due to the short duration of this experiment, which involved only 7 days of leptin infusion, compared with the 5-day reproductive cycle of mice. Either this period of leptin infusion was not long enough to stimulate the growth of reproductive tissue, or early stages of cell development, which would have been detected by histological examination of the tissue, were not apparent as a significant change in tissue weight.

Measurements of catecholamines and monoamines in several brain areas of lean and *ob/ob* mice demonstrated many genotypic differences. Although NE was elevated in both the hypothalamus and brain stem of *ob/ob* mice compared with levels in lean mice, NE metabolism (MHPG/NE) was lower in the hypothalamus of *ob/ob* mice than lean mice, confirming previous reports of reduced NE synthesis and metabolism in these animals (47). The failure of leptin to reverse this defect demonstrates that it is not a direct result of leptin deficiency and is not associated with the hyperphagia, hyperinsulinemia, or hypothermia. DA metabolism, measured as DOPAC/DA or HVA/DA, was elevated in *ob/ob* mice compared with lean animals. The lack of site specificity and the failure of leptin to correct this difference suggest that it is also unrelated to the energy balance aspects of leptin deficiency. 5-HT and its metabolism, indicated by the 5-HIAA/5-HT ratio, was elevated in *ob/ob* mice and responded to leptin

TABLE 7. Brain catecholamines in lean and *ob/ob* mice

	Brain stem			Hypothalamus			Frontal cortex		
	Lean	*ob/ob*	Significance	Lean	*ob/ob*	Significance	Lean	*ob/ob*	Significance
NE	0.81 ± 0.01	0.91 ± 0.02	0.0001 (32,1)	1.46 ± 0.03	1.68 ± 0.04	0.0001 (26,1)	0.46 ± 0.02	0.48 ± 0.02	NS
MHPG	0.12 ± 0.01	0.13 ± 0.01	NS	0.79 ± 0.03	0.77 ± 0.03	NS			
MHPG/NE	0.15 ± 0.01	0.14 ± 0.01	NS	0.54 ± 0.02	0.46 ± 0.02	0.006 (8.3,1)			
DA	0.072 ± 0.002	0.080 ± 0.003	0.04 (4.6,1)	0.30 ± 0.01	0.28 ± 0.01	NS	0.089 ± 0.005	0.094 ± 0.007	NS
DOPAC	0.053 ± 0.001	0.040 ± 0.002	0.001 (36,1)	1.30 ± 0.08	1.43 ± 0.12	NS	0.079 ± 0.010	0.060 ± 0.04	NS
HVA	0.090 ± 0.002	0.109 ± 0.006	0.001 (12,1)	0.47 ± 0.01	0.54 ± 0.02	0.01 (6.5,1)	0.109 ± 0.004	0.143 ± 0.008	0.0003 (15,1)
DOPAC/DA	0.80 ± 0.04	0.53 ± 0.04	0.0001 (24,1)	0.25 ± 0.01	0.23 ± 0.01	NS	0.88 ± 0.07	0.67 ± 0.04	0.01 (7.2,1)
HVA/DA	1.31 ± 0.04	1.40 ± 0.07	NS	1.63 ± 0.03	1.93 ± 0.06	0.0001 (19,1)	1.29 ± 0.05	1.62 ± 0.07	0.0005 (14,1)

Data are the mean ± SEM for groups of 36 lean or *ob/ob* mice. The significance is for a genotype effect on catecholamine concentration determined by two-way ANOVA in which genotype and leptin dose were independent variables. There was no significant effect of leptin or of an interaction between genotype and leptin on any of the parameters; therefore, the means for the two genotypes are the average from all animals in the experiment.

treatment. Doses of leptin as low as 2 μg/day leptin increased the 5-HIAA concentration and 5-HT metabolism in the hypothalamus and brain stem, but not the cortex, of *ob/ob*, but not lean, mice. The site specificity of this response suggests an association between the reduced food intake in leptin-treated *ob/ob* mice and 5-HT, which is known to suppress food intake (48, 49). It is possible that the satiety effect of leptin in *ob/ob* mice is mediated in part by modulation of this neurotransmitter, whereas the absence of a change in 5-HT metabolism in leptin-treated lean mice is consistent with their recovery of a normal food intake by the end of the experiment. To date, the majority of studies investigating leptin-sensitive central control of food intake have focused on neuropeptide Y, which is elevated in the hypothalamus of genetically obese mice and rats and is down-regulated by leptin (38, 50). However, neuropeptide Y knockout mice are responsive to leptin (51), indicating some redundancy in the mechanisms that mediate leptin-induced hypophagia. The results of this experiment demonstrate a correlation between 5-HT metabolism and food intake in *ob/ob* mice, but further investigation is needed to establish the true relationship among leptin, 5-HT, and food intake.

The second objective of this study was to determine genotypic differences in response to leptin in lean and *ob/ob* mice. The most obvious difference was an exaggerated sensitivity to leptin in *ob/ob* mice compared with that in lean animals. In *ob/ob* mice, several changes were observed with 2 μg/day leptin, whereas only the two highest doses of leptin caused reliable changes in lean mice, consistent with other reports of no response in lean animals to doses of leptin that produce significant changes in food intake, body weight, serum insulin, and body temperature in *ob/ob* mice (9). Potential explanations for the increased sensitivity of *ob/ob* mice to the protein include a difference in circulating concentrations of protein; a decreased amount of binding protein in *ob/ob* mice, resulting in an increased amount of bioavailable protein in the circulation; an increased number of leptin receptors in *ob/ob* mice; or a failure of obese mice to down-regulate the receptor in response to continuous agonism by leptin. There was no obvious difference in circulating concentrations of leptin in lean and *ob/ob* mice given the same dose of protein, indicating that increased responsiveness in *ob/ob* mice could not be attributed to elevated levels of circulating protein. We were not able to determine the proportion of circulating leptin that was bound to either binding proteins (16) or soluble receptor (11), and the possibility of an increased amount of free leptin in *ob/ob* mice cannot be excluded. In this experiment we only measured short form receptors present in the liver. There was no effect of leptin treatment on the amount of receptor present in either lean or obese mice, and the Western blots suggested that more receptor was present in tissue from lean than *ob/ob* mice.

In summary, the results of this experiment, in which a large number of variables were measured in lean and obese mice treated with increasing amounts of leptin, demonstrated that *ob/ob* mice were more responsive to leptin than were lean animals; they showed reduced food intake, body weight, and serum insulin and glucose levels and increased hypothalamic and brain stem serotonin metabolism when given 2 μg/day leptin. The small amount of protein needed

to initiate these changes suggests that they are all primary physiological responses to leptin. As human and mouse leptin have 84% homology (3), it is likely that even lower concentrations of murine recombinant leptin would be required to initiate a response in *ob/ob* mice. Correction of hypothermia and increased expression of brown fat UCP in *ob/ob* mice required relatively large doses of protein, which suggests that these effects are representative of responses to a pharmacological dose of leptin. In contrast to *ob/ob* mice, the only response observed in lean mice was a transient reduction in food intake and a reduction in body weight of mice given the two highest doses of leptin. The absence of leptin in *ob/ob* mice during growth and development may cause them to be especially sensitive to exogenous protein and result in a failure to adapt to the protein, such that mechanisms that prevent a continued effect of leptin on food intake in lean mice are absent or minimal in *ob/ob* mice. In this experiment we did not determine which of the changes in leptin-treated *ob/ob* mice were a direct response to the protein and which were secondary to the state of negative energy balance that resulted from a sustained inhibition of food intake. The exaggerated sensitivity of *ob/ob* mice to leptin indicates that other animal models, such as dietary obese mice, are more appropriate when considering the effect of leptin on physiological and biochemical parameters *in vivo*.

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