Transient Effects of Long-Term Leptin Supplementation in the Prevention of Diet-Induced Obesity in Mice

Richard S. Surwit, Christopher L. Edwards, Suma Murthy, and Ann E. Petro

Low plasma leptin levels have been shown to be associated with the development of obesity in mice as well as in humans. The present study was undertaken to determine if raising plasma leptin levels of obesity-prone C57BL/6J (B6) mice to those seen in obesity-resistant A/J mice would prevent the development of diet-induced obesity. Four-week-old B6 (n = 40) and A/J (n = 10) male mice were weaned onto a low-fat (11% kcal) diet. When the animals weighed 20 g, their diets were changed to a high-fat (HF) diet (58% kcal), and a continuous infusion of leptin (0.4 mg · kg^{-1} · day^{-1}) or phosphate-buffered saline (control) was started using Alzet minipumps. The A/J mice were not treated but were included to monitor the efficacy of the minipumps in raising plasma leptin in B6 mice. The mice were followed for 12 weeks. Chronic treatment with leptin for 4 weeks raised plasma levels in B6 mice to that of A/J mice. Plasma leptin in B6 control mice remained significantly lower than A/J mice through week 4. By week 8, leptin levels in the B6 control group had risen and were similar to A/J mice. Although there were significant weight differences between B6 treated and B6 control groups for 2–3 weeks after pump implantation, these differences were transient. Ultimately, there were no weight differences between the B6 treated and B6 control groups. There were no differences in plasma glucose between B6 treated and control groups. Plasma insulin values were also not different between the 2 groups. There was no effect of leptin supplementation on locomotor activity or food intake in B6 mice. In summary, this study demonstrates that leptin supplementation in animals that show low plasma leptin levels in response to fat feeding may slow but does not prevent the subsequent development of diet-induced obesity. *Diabetes* 49:1203–1208, 2000

T he discovery of leptin and its receptor has revealed a new hormonal feedback system that may contribute substantially to the regulation of body weight (1–4). Leptin has been demonstrated to influence body weight through effects on metabolic rate as well as on food intake, oxygen consumption, and increased body temperature (5–8). Mutations in the *obese* gene, which encodes leptin, lead to hyperphagia and severe obesity in animals (9) and humans (10,11). In these cases (5,6,12), treatment with exogenous leptin reduced body weight and eliminated the hyperphagia associated with the absence of leptin. Levin et al. (13) extended these studies and, using a pair feeding design, showed that in *ob/ob* mice the decreases in body weight and fat-pad weight as a result of exogenous leptin were independent of decreases in food intake. In addition, Sindelar et al. (14) reported that when diabetes was induced in rats using streptozotocin, plasma leptin fell and hyperphagia ensued. Leptin supplementation prevented the increased food intake in this model. However, most human obesity is associated with increased circulating leptin (15–17), leading to the theory that, like type 2 diabetes, obesity involves resistance to the action of a protein hormone (18,19) at some unknown site.

Little is known about the role leptin plays in the etiology of obesity in nonmutant animals or humans. The relationship between plasma leptin levels and subsequent weight gain in humans is not clear. Interestingly, some studies demonstrate that relatively low plasma leptin levels predict subsequent obesity in human populations (20–23). Plasma leptin levels were shown to predict weight gain in a sample of 36 Pima Indians followed over 3 years. Those individuals with higher leptin levels adjusted for percent body fat remained at a stable weight, whereas those with lower leptin levels gained weight (20). In prepubertal girls, but not boys, leptin levels predicted subsequent changes in percent body fat (23). Matkovic and colleagues (21,22) have confirmed the relationship between low plasma leptin and subsequent obesity in young Caucasian females. However, other studies are not confirmatory. A longitudinal study done in Mauritius found no relationship between plasma leptin levels and subsequent weight (24). Chessler et al. (25) found a positive relationship between leptin levels and fat accumulation in Japanese Americans.

Although the effect of diet was not examined in these studies, several investigations have reported that fasting plasma leptin levels were not affected by dietary fat (26,27). However, in a recent report, Havel et al. (28) demonstrated that high-fat meals decrease 24-h circulating leptin when compared with high-carbohydrate meals. The plasma leptin nighttime peak and the 24-h area under the curve were significantly lowered by a high-fat diet. This led to the suggestion that decreases in leptin secretion would reduce the overall amount of leptin available to the central nervous system and thus contribute to the adipogenic effects of high-fat diets.

The C57BL/6J (B6) mouse is a well-characterized model of diet-induced diabetes and obesity. Additionally, it is the back-

ground strain on which the *ob/ob* mutation is commonly placed. The B6 mouse remains lean and otherwise normal on low-fat diets; but when raised on a high-fat diet, it develops severe obesity, hyperglycemia, hyperinsulinemia, β-cell dysfunction, and hypertension in adulthood (29–31). B6 mice show both increased caloric intake and increased feed efficiency (body weight gain per kilocalorie consumed) in response to fat (30). In contrast, strains such as A/J gain weight on a high-fat diet in direct proportion to increased caloric intake but maintain normal glucose and insulin levels. The syndrome develops in B6 mice fed high fat in spite of the fact that they are 2–3 times more active than A/J mice (32).

Recently, we identified differential changes in leptin levels in A/J and B6 mice in response to a high-fat diet. At 4 weeks of age, the B6 mouse has significantly lower plasma leptin levels when compared with A/J mice. Within 1 month of being fed a high-fat diet, A/J animals show a 2-fold rise in plasma leptin that plateaus and remains constant even as animals gain weight. B6 animals lack this response to diet and show increased levels of leptin only after the development of obesity. Lower plasma leptin levels in B6 mice when compared with A/J can be observed for up to 10 weeks after introduction of a high-fat diet. The leptin levels in B6 mice reach and exceed those of A/J only after B6 animals develop massive obesity (body weight >40 g). Low plasma leptin may help explain the propensity of B6 mice to develop diet-induced obesity (33).

The present study was undertaken to gain understanding of the role of leptin in the etiology of diet-induced diabetes and obesity. The hypothesis that leptin supplementation would prevent diet-induced diabetes and obesity in B6 mice was tested.

RESEARCH DESIGN AND METHODS

Animals. Forty B6 and 10 A/J male mice were received at 4 weeks of age from Jackson Laboratories and were housed 5 animals per cage in a temperature-controlled vivarium equipped with a 12-h light/dark cycle (lights on 0700). The mice were fed a low-fat (11% kcal) diet (Research Diets, New Brunswick, NJ). When the animals weighed 20 g, the diet was changed to high fat (58% kcal). In addition, a continuous infusion of phosphate-buffered saline or leptin (0.4 mg · kg^{-1} · day^{-1}) was started using Alzet osmotic minipumps (model 2004).

Surgical procedures. The minipumps were filled with either recombinant mouse leptin (kindly provided by Amgen) or phosphate-buffered saline and implanted subcutaneously on the dorsal surface of the mouse. The concentration of leptin was based on previous dose-response studies (data not shown). The animals were anesthetized using 60 mg/kg ketamine and 0.2 mg/kg medetomidine. Sedation was reversed with 1 mg/kg atipamezole.

The mice were followed for 12 weeks. The model 2004 minipump delivers solutions for 28 days. Therefore, the pumps were implanted at week 0 and replaced at weeks 4 and 8. When the pumps were explanted, they were examined to ensure that some of the solution remained in the pump. The concentration of leptin was recalculated based on the weight of the mice at the time of replacement. Twenty B6 mice were assigned to each treatment group. The A/J were included but not treated to monitor the efficacy of the minipumps in raising plasma leptin in B6 mice.

Body weight and food intake. Body weight and food intake were measured daily for the first 5 days following surgery. Thereafter, body weight was measured weekly and food intake twice weekly.

Feed efficiency. The feed efficiency (grams body weight gain divided by calories consumed) was calculated on a cage basis.

Sample collection. Blood samples were collected 5 days before the treatment began and 24–26 days after each pump implantation procedure. Food was withheld for 8 h before sample collection. The samples were collected from the retro-orbital sinus cavity in nonanesthetized animals.

Core temperature. Temperature measurements were made 2 h after the beginning of the light cycle (0900) at 2 time points in the study. The first measurement was made 4 days before the treatment began, and the second assessment of temperature was made 1 month after the treatment started. Rectal temperatures were taken with a thermocouple microprobe (Physitemp, Clifton, NJ). The probe was inserted 10 mm into the rectum, and the temperature was recorded after 15 s.
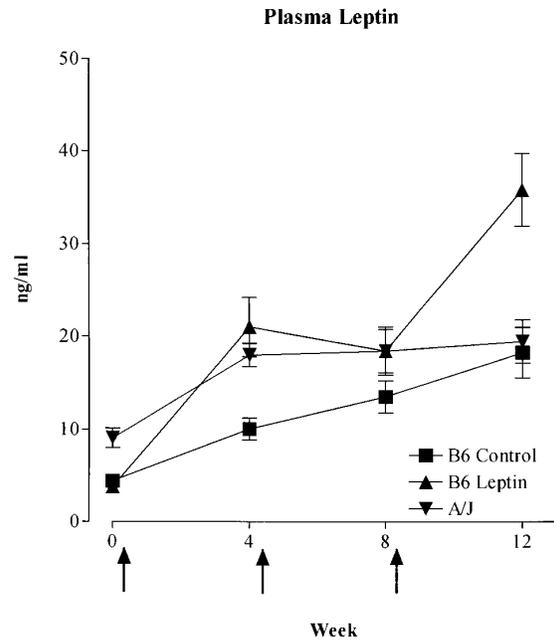

FIG. 1. The effect of continuous leptin infusion (0.4 mg · kg^{-1} · day^{-1}) on plasma leptin. The arrows indicate the time minipumps were implanted. Data are means ± SE. Significant differences are described in the text.

Locomotor activity. The locomotor activity of the animals was measured using the Opti-Varimex Activity Meter (Columbus Instruments International, Columbus, OH). Briefly, the apparatus is a Plexiglas box surrounded by a 15 by 15 grid of infrared beams spaced 1 inch apart. Locomotion was measured 3–5 days before the treatment started and 1 month after the treatment was underway. Mice to be tested were transferred to the testing room in their home cages and left undisturbed for 1 h before the 1-h test period. The animal was then placed in the Plexiglas chamber and left uninterrupted for the test period. Three days were required to complete the testing of all animals. Three animals, 1 animal from each treatment, were tested in each test period. The data are reported as the total number of beams broken in 1 h.

Biochemical analyses. Plasma samples were analyzed for glucose, insulin, and leptin concentration. Glucose was determined using the glucose oxidase method (Beckman Glucose Analyzer II). Insulin and leptin were measured using double antibody radioimmunoassay kits (Linco Research, St. Louis, MO). The insulin assay is based on a rat standard, and the leptin assay is based on a mouse standard.

Fat pad weight and adiposity index. At the termination of the study, the animals were killed, and the epididymal, inguinal, retroperitoneal, and mesenteric fat pads were dissected using anatomical landmarks and weighed. The gastrointestinal tract was removed and the eviscerated mouse weighed. The adiposity index was calculated as the total fat pad weight divided by the weight of the eviscerated mouse.

Statistical analysis. The data are expressed as means ± SE. Data were analyzed by analysis of variance. Although the B6 mice were randomly assigned to either a control or leptin-treated group, it was found that the pretreatment means for insulin and temperature were different between the 2 groups. These data were analyzed using analysis of covariance (ANCOVA). Pretreatment values were used as the covariate. Comparisons between B6 control and leptin-treated groups were made using the *t* test for independent samples.

RESULTS

Plasma leptin. As previously observed, B6 mice have lower plasma leptin levels than do A/J at baseline (B6 = 4.11 ± 0.27 ng/ml vs. A/J = 7.64 ± 0.69 ng/ml; *P* < 0.0001). In addition, A/J mice showed their characteristic plasma leptin response after the introduction of a high-fat diet, i.e., a rapid rise that reached a plateau and remained constant. As shown in Fig. 1, chronic treatment with leptin was effective in raising plasma leptin concentration in B6 mice to levels similar to those of
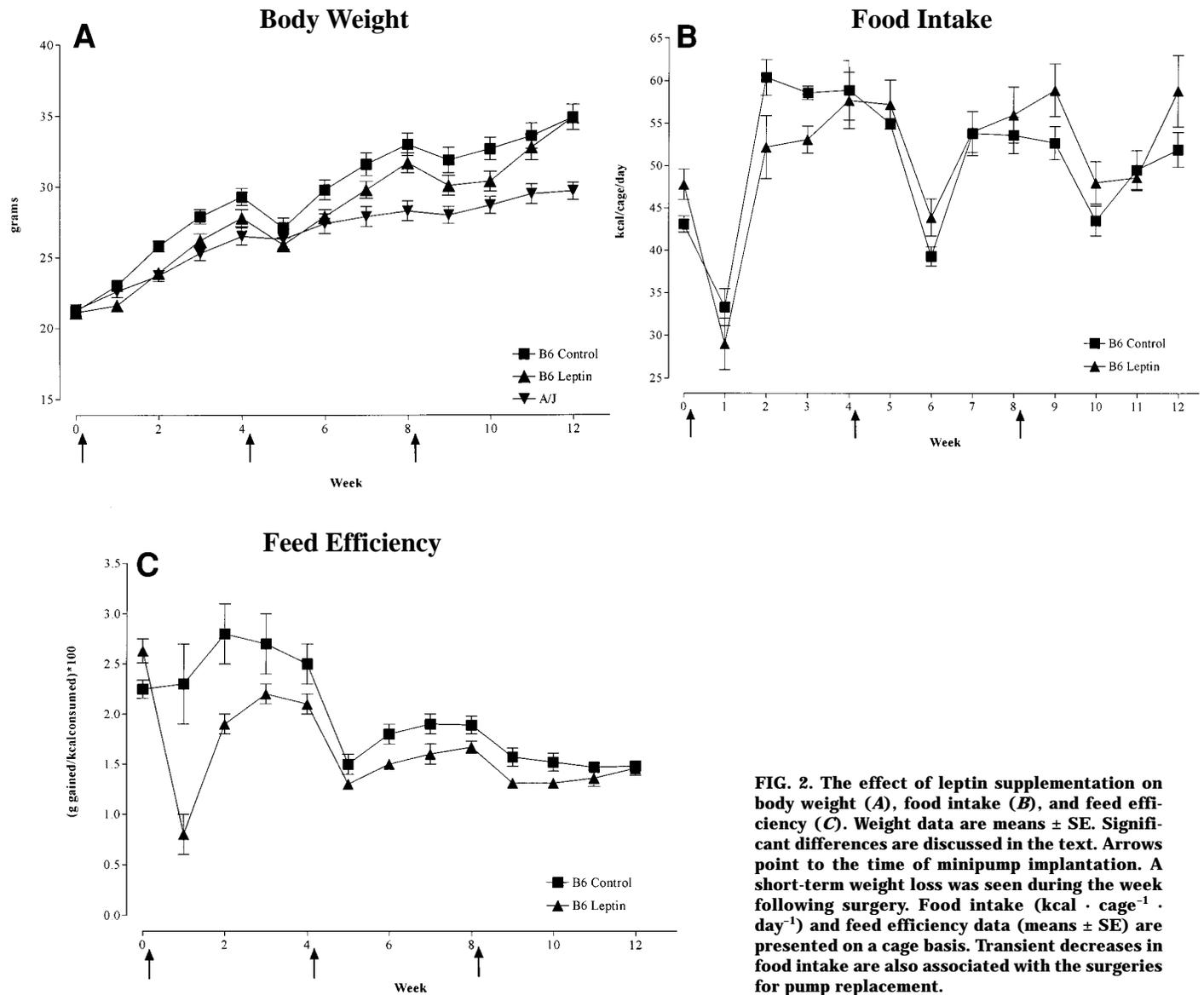
Body Weight

A

Food Intake

B

Feed Efficiency

C

FIG. 2. The effect of leptin supplementation on body weight (*A*), food intake (*B*), and feed efficiency (*C*). Weight data are means ± SE. Significant differences are discussed in the text. Arrows point to the time of minipump implantation. A short-term weight loss was seen during the week following surgery. Food intake (kcal · cage^{-1} · day^{-1}) and feed efficiency data (means ± SE) are presented on a cage basis. Transient decreases in food intake are also associated with the surgeries for pump replacement.

A/J mice. Furthermore, until week 8, the plasma leptin in B6 control mice remained significantly lower when compared with A/J mice. By week 8, leptin levels in the B6 control group had risen and were similar to A/J mice (B6 control 13.47 ± 1.72 ng/ml vs. A/J 18.42 ± 2.30 ng/ml; $P = 0.11$) (Fig. 1). **Body weight.** Chronic infusion of leptin for 12 weeks did not prevent obesity in the B6 mouse. Initially, leptin suppressed weight gain in B6 mice. However, this effect was transient. Ultimately, there were no weight differences between the B6 leptin-treated and B6 control groups ($P = 0.98$) (Fig. 2*A*). B6 mice were significantly heavier than A/J mice (B6 34.9 ± 0.6 g vs. A/J 29.7 ± 0.6 g; $P < 0.001$). Weight loss depicted in Fig. 2*A* is associated with surgery for pump implantation. **Food intake.** Food intake measurements were made twice weekly. The food is pelleted and measurements of intake are generally easy. However, A/J mice shredded the food into tiny particles that could not be recovered efficiently. The food intake for this strain is unreliable and therefore has not been included. This had not been observed previously. Food intake data for B6 mice are shown in Fig. 2*B*. Decreases in

food intake were associated with surgeries. Food intake was not affected by treatment.
Feed efficiency. As with body weight, feed efficiency was significantly depressed in the B6 leptin-treated mice when compared with B6 control mice for 2–3 weeks following the surgical procedure. However, these differences were transient and were not significant at 12 weeks ($P = 0.77$) (Fig. 2*C*).
Plasma glucose and insulin. Leptin supplementation did not prevent the development of diabetes in the B6 mouse. Plasma glucose levels were similar between A/J and B6 mice at baseline ($P = 0.81$). Plasma glucose levels of control and leptin-treated B6 mice rose significantly within 4 weeks ($P < 0.01$ in both groups). Values for control animals were 9.3 ± 0.2 mmol/l (time 0) versus 10.7 ± 0.7 mmol/l (time 4 weeks). For leptin-treated mice, values were 8.9 ± 0.1 mmol/l (time 0) versus 10.7 ± 0.3 mmol/l (time 4 weeks). In addition, by week 4, the glucose levels in B6 mice were significantly higher when compared with those of A/J mice ($P < 0.0001$). Furthermore, there were no differences between the B6 control and treated groups at weeks 8 and 12 ($P > 0.05$ in both groups at both time
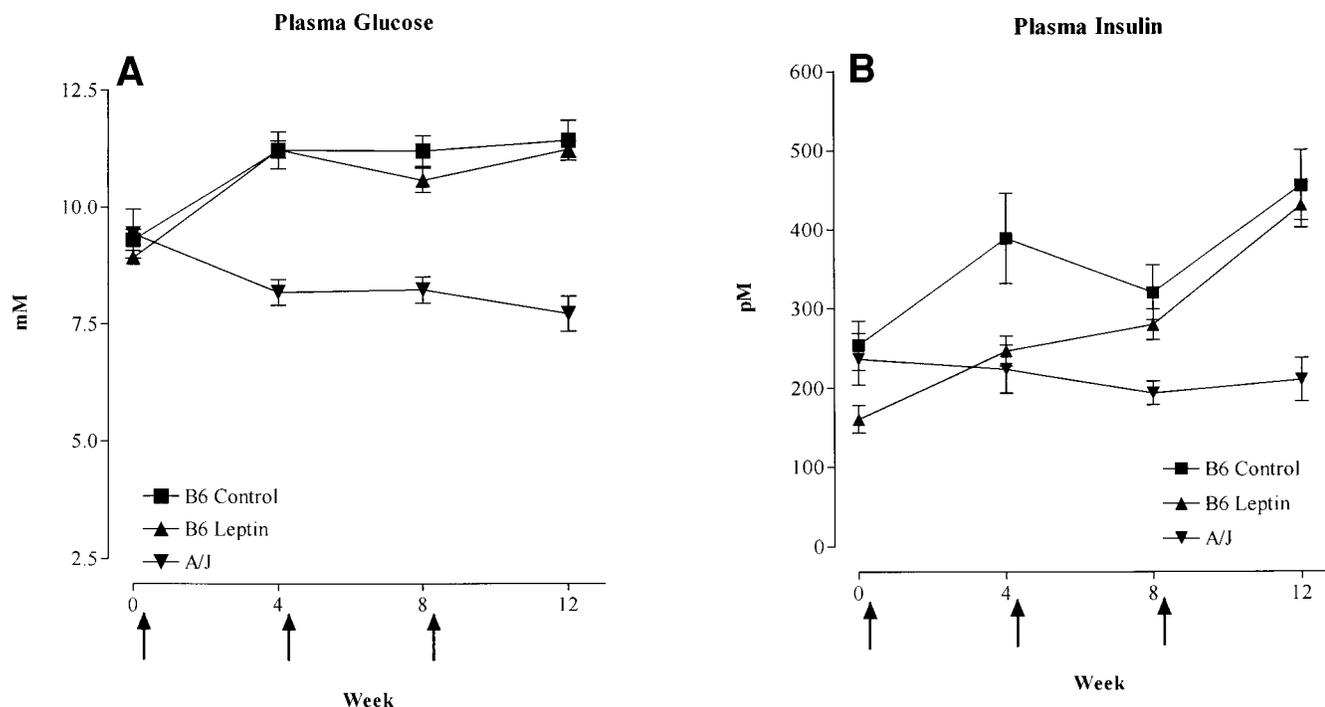
FIG. 3. The effect of leptin supplementation on plasma glucose (*A*) and insulin (*B*). Data are means ± SE. Significant results are described in the text. The arrows indicate the time Alzet minipumps were implanted.

points). The B6 mice remained hyperglycemic throughout the study (Fig. 3*A*).

There was a significant strain × time interaction (*P* < 0.001) for plasma insulin levels. Insulin levels in B6 mice rose significantly throughout the study, whereas levels in A/J mice remained similar to baseline. Although the B6 mice were randomly assigned to either a control or leptin-treated group, it was found that the pretreatment means for insulin were different between the 2 groups. Therefore, these data were analyzed using ANCOVA. Pretreatment values were used as the covariate. This statistical analysis showed no difference between B6 control and leptin-treated mice at any time during the experiment (Fig. 3*B*).

Obesity. Leptin supplementation did not prevent obesity in the B6 mouse. None of the differences in fat pad weights were statistically significant. The adiposity index (sum of fat pad weights divided by the eviscerated weight of the animal) was also not different between B6 control and leptin-treated groups (Table 1). The adiposity index in both groups of B6 mice was lower when compared with that in A/J mice. This result was unexpected and was presumed to be a consequence of the surgical procedures performed on the B6 mice. This is supported by previous unpublished data that did not involve surgical procedures for which the adiposity indexes of B6 and A/J mice were 0.207 ± 0.037 and 0.195 ± 0.011.

Locomotor activity. There was no effect of leptin supplementation on locomotor activity in B6 mice as assessed by the number of infrared beams broken in a 15 by 15 grid in 1 h (control 19,815 ± 1,051 vs. leptin 21,346 ± 782; *P* = 0.15). However, B6 mice were ~3 times more active than A/J mice (Table 1). Because, after 1 month of treatment, there were no differences in locomotor activity between B6 control and leptin groups, further measurements were not conducted.

Body temperature. Initially, B6 mice had a lower core temperature than did A/J mice (B6 36.7 ± 0.14°C vs. A/J 37.5 ± 0.14°C; *P* < 0.001). At the end of the first month of treatment, there was a trend toward increased body temperature in the leptin-treated B6 mice (control 35.8 ± 0.13°C vs. leptin-treated 36.3 ± 0.10°C [*P* = 0.054]). At the end of the second month, the core temperature of the B6 control mice was not different from the that of the leptin-treated mice (data not shown). The cohort of B6 mice assigned to the leptin treatment had higher pretreatment temperatures than did the cohort assigned to the control group; therefore, ANCOVA was used for the post-treatment analysis. Pretreatment values were the covariate (Table 1).

Effect of surgery. The incisions in a number of mice, particularly in the control group, did not heal well and had to be resutured. These mice were not included in the data analysis; therefore, *n* = 12 for B6 control mice and *n* = 19 for leptin-treated mice. It is not known whether this was an effect of the leptin treatment on wound healing. However, Ring et al. (34) recently demonstrated that wound healing was accelerated in *ob/ob* mice when leptin was administered systemically and topically. Additionally, Sierra-Honigmann et al. (35) found that the leptin receptor (Ob-R) is expressed in the vasculature and that leptin induces angiogenesis in vitro and in vivo.

DISCUSSION

Mutations in the leptin gene are clearly responsible for severe obesity and diabetes in mice and humans (1,10). However, such mutations are relatively rare in nature and do not contribute significantly to the etiology of human obesity. Recently, several reports of a relationship between relative levels of circulating leptin and the tendency to develop obesity have been reported in humans and in rodents (14,20–23,33). In the current study, we attempted to prevent diet-induced obe-

TABLE 1
The effect of continuous infusion of leptin for 12 weeks on obesity, locomotion, and temperature

	B6 saline	B6 leptin	A/J
n	12	19	10
Obesity measurements			
Fat pad weight (g)			
Epididymal	1.85 ± 0.14	1.60 ± 0.14	1.60 ± 0.10
Mesenteric	0.58 ± 0.07	0.46 ± 0.05	0.51 ± 0.04
Retroperitoneal	0.42 ± 0.03	0.37 ± 0.03	0.43 ± 0.04
Inguinal	1.29 ± 0.14	0.99 ± 0.10	1.24 ± 0.09
Eviscerated weight	29.9 ± 0.76	29.6 ± 0.61	23.9 ± 0.45
Adiposity index	0.13 ± 0.009	0.11 ± 0.008	0.16 ± 0.008
Temperature (C°)			
Pretreatment	36.3 ± 0.2	37.1 ± 0.1	37.5 ± 0.1
Posttreatment	35.8 ± 0.1	36.3 ± 0.1	36.0 ± 0.1
Locomotion			
Pretreatment	23,270 ± 644	21,588 ± 726	5,623 ± 759
Posttreatment	19,185 ± 1,051	21,346 ± 782	5,654 ± 837

Data are *n* or means ± SE. The adiposity index is the sum of the fat pad weights divided by the weight of the eviscerated mouse. Locomotion is the number of infrared beams in a square grid of 15 beams spaced 1 inch apart broken in 1 h.

sity and diabetes by raising the level of leptin in the obesity/diabetes-prone B6 mouse to the level naturally observed in the obesity/diabetes-resistant A/J strain. Leptin supplementation over the first 4 weeks raised the levels of plasma leptin in B6 mice to those of unsupplemented A/J mice and appeared to attenuate diet-induced obesity by decreasing feed efficiency. However, the effect of leptin on weight disappeared by the end of the experiment, despite the fact that at 12 weeks leptin levels in supplemented B6 mice were higher than levels in all other groups. This apparent tachyphyllaxis to continuous leptin dosing has not previously been demonstrated. Although leptin supplementation produced a modest transient decrease in weight gain and feed efficiency in B6 mice, these effects also disappeared after ~5 weeks of treatment, and there was no long-term effect of leptin supplementation in preventing diet-induced obesity or diabetes in the B6 mouse. Contrary to some reports in the literature, animals receiving leptin supplementation actually increased their food consumption relative to controls. Thus, the effect of leptin is similar to that of a β_3AR agonist in this animal model (36), suggesting that leptin has more of an effect on energy expenditure than on energy intake (Fig. 2*B*).

Although leptin replacement in the leptin-deficient *ob/ob* mouse will reverse the hyperglycemia and hyperinsulinemia characteristic of this model (5), leptin supplementation in fat-fed B6 mice had no effect on the development of elevated plasma glucose or insulin. This finding suggests that it is doubtful that the difference observed in leptin levels between B6 and A/J mice following the introduction of a high-fat diet is responsible for the hyperglycemia and hyperinsulinemia that subsequently develop in B6 mice. Thus, although an absolute leptin deficiency can cause diabetes, it is unlikely that relative differences in plasma leptin observed in normal individuals are related to the predisposition to develop diabetes.

As in animal studies, leptin supplementation in obese humans with a normal leptin gene has produced only modest effects on body weight. Subjects receiving subcutaneous injections of leptin (0.3 mg/kg) on a daily basis lost 7.1 kg over 6 months, whereas controls lost only 1.7 kg (37). Friedman

and Halaas (38) recently raised the possibility that individuals with relatively low leptin might be more sensitive to the weight-reducing properties of leptin supplementation. Our results clearly show that, at least in mice, this hypothesis is not correct.

In summary, this study demonstrates that leptin supplementation delays, but does not prevent, the development of diet-induced obesity in animals with low plasma leptin. Plasma leptin levels in nonmutant animals do not appear to be causally related to the development of diet-induced obesity. This finding casts doubt on the notion that leptin therapy for obesity or diabetes might be more effective in individuals with relatively low levels of this hormone.

REFERENCES
1. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman J: Positional cloning of the mouse *obese* gene and its human homologue. *Nature* 372:425–432, 1994
2. Tartaglia LA, Dembski M, Weng X, Deng N, Culpepper J, Devos R, Richards GJ, Campfield LA, Clark FT, Deeds J, Muir C, Sanker S, Moriarty A, Moore KJ, Smutko JS, Mays GG, Woolf EA, Monroe CA, Tepper RI: Identification and expression cloning of a leptin receptor, OB-R. *Cell* 83:1263–1271, 1995
3. Lee GH, Proenca R, Montez JM, Carroll KM, Darvishzadeh JG, Lee JI, Friedman JM: Abnormal splicing of the leptin receptor in *diabetic* mice. *Nature* 379:632–635, 1996
4. Chen H, Charlat O, Tartaglia LA, Woolf EA, Weng X, Ellis SJ, Lakey ND, Culpepper J, Moore KJ, Breitbart RE, Duyk GM, Tepper RI, Morgenstern JP: Evidence that the diabetes gene encodes the leptin receptor: identification of a mutation in the leptin receptor gene in *db/db* mice. *Cell* 84:491–495, 1996
5. Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T, Collins F: Effects of the *obese* gene product on body weight regulation in *ob/ob* mice. *Science* 269:540–543, 1995
6. Halaas JL, Gajiwala KS, Maffei M, Cohen SL, Chait BT, Rabinowitz D, Lallone RL, Burley SK, Friedman JM: Weight-reducing effects of the plasma protein encoded by the *obese* gene. *Science* 269:543–546, 1995
7. Collins S, Kuhn CM, Petro AE, Swick AG, Chrunyk BA, Surwit RS: Role of leptin in fat regulation (Letter). *Nature* 380:677, 1996
8. Campfield LA, Smith FJ, Guisez Y, Devos R, Burn P: Recombinant mouse OB

protein: evidence for a peripheral signal linking adiposity and central neural networks. *Science* 269:546–549, 1995

9. Bray GA, York DA: Hypothalamic and genetic obesity in experimental animals: an autonomic and endocrine hypothesis. *Physiol Rev* 59:719–809, 1979

10. Strobel A, Issad T, Camoin L, Ozata M, Strasbourg AD: A leptin missense mutation associated with hypogonadism and morbid obesity. *Nat Gen* 18:213–215, 1998

11. Montague CT, Farooqi IS, Whitehead JP, Soos MA, Rau H, Wareham NJ, Sewter CP, Digby JE, Mohammed SN, Hurst JA, Cheetham CH, Earley AR, Barnett AH, Prins JB, O'Rahilly S: Congenital leptin deficiency is associated with severe early-onset obesity in humans. *Nature* 387:903–908, 1997

12. Farooqi IS, Jebb SA, Langmack G, Lawrence E, Cheetham CH, Prentice AM, Hughes IA, McCamish MA, O'Rahilly S: Effects of recombinant leptin therapy in a child with congenital leptin deficiency. *N Engl J Med* 341:879–883, 1999

13. Levin N, Nelson C, Gurney A, Vandlen R, DeSauvage F: Decreased food intake does not completely account for adiposity reduction after ob protein infusion. *Proc Natl Acad Sci U S A* 93:1726–1730, 1996

14. Sindelar DK, Havel PJ, Seeley RJ, Wilkinson CW, Woods SC, Schwartz MW: Low plasma leptin levels contribute to diabetic hyperphagia in rats. *Diabetes* 48:1275–1280, 1999

15. Maffei M, Halaas J, Ravussin E, Pratley RE, Lee GH, Zhang Y, Fei H, Kim S, Lallone R, Ranganathan S, Kern PA, Friedman JM: Leptin levels in human and rodent: measurement of plasma leptin and *ob* RNA in obese and weight-reduced subjects. *Nat Med* 1:1155–1161, 1995

16. Considine RV, Sinha MK, Heiman ML, Kriauguinas A, Stephens TW, Nyce MR, Ohannesian JP, Marco CC, McKee LJ, Bauer TL, Caro JF: Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med* 334:292–295, 1996

17. Lonnqvist F, Arner P, Nordfors L, Schalling M: Overexpression of the obese *(ob)* gene in adipose tissue of human obese subjects. *Nat Med* 1:950–953, 1995

18. Hamilton BS, Paglia D, Kwan AYM, Deitel M: Increased *obese* mRNA expression in omental fat cells from massively obese humans. *Nat Med* 1:953–956, 1995

19. Frederich RC, Hamann A, Anderson S, Lollmann B, Lowell BB, Flier J: Leptin levels reflect body lipid content in mice: evidence for diet-induced resistance to leptin action. *Nat Med* 1:1311–1314, 1995

20. Ravussin E, Pratley RE, Maffei M, Wang H, Friedman JM, Bennett PH, Bogardus C: Relatively low plasma leptin concentrations precede weight gain in Pima Indians. *Nat Med* 3:238–240, 1997

21. Matkovic V, Ilich JZ, Badenhop NE, Skugor M, Clairmont A, Klisovic D, Landoll JD: Gain in body fat is inversely related to the nocturnal rise in serum leptin level in young females. *J Clin Endocrinol Metab* 82:1368–1372, 1997

22. Matkovic V, Ilich JZ, Skugor M, Badenhop NE, Goel P, Clairmont A, Klisovic D, Nahhas RW, Landoll JD: Leptin is inversely related to age at menarche in human females. *J Clin Endocrinol Metab* 82:3239–3245, 1997

23. Ahmed ML, Ong KKL, Morrell DJ, Cox L, Drayer N, Perry L, Preece MA, Dunger DB: Longitudinal study of leptin concentrations during puberty: sex differences and relationship to changes in body composition. *J Clin Endocrinol Metab* 84:899–905, 1999

24. Hodge AM, de Courten MP, Dowse GK, Zimmet PZ, Collier GR, Gareeboo H, Chitson P, Fareed D, Hemraj F, Alberti KGMM, Tuomilehto J: Do leptin levels predict weight gain? A 5-year follow-up study in Mauritius. *Obes Res* 6:319–325, 1996

25. Chessler SD, Fujimoto WY, Shofer JB, Boyko EJ, Weigle DS: Increased plasma leptin levels are associated with fat accumulation in Japanese Americans. *Diabetes* 47:239–243, 1998

26. Weigle DS, Duell PB, Connor WE, Steiner RA, Soules MR, Kuijper JL: Effect of fasting, refeeding, and dietary fat restriction on plasma leptin levels. *J Clin Endocrinol Metab* 82:561–565, 1997

27. Schrauwen P, van Marken Lichtenbelt WD, Westerterp KR, Saris WHM: Effect of diet composition on leptin concentration in lean subjects. *Metabolism* 46:420–424, 1997

28. Havel PJ, Townsend R, Chaump L, Teff K: High-fat meals reduce 24-h circulating leptin concentrations in women. *Diabetes* 48:334–341, 1999

29. Surwit RS, Kuhn CM, Cochrane C, McCubbin JA, Feinglos MN: Diet-induced type II diabetes in C57BL/6J mice. *Diabetes* 37:1163–1167, 1988

30. Surwit RS, Feinglos MN, Rodin J, Sutherland A, Petro AE, Opara EC, Kuhn CM, Rebuffe-Scrive M: Differential effects of fat and sucrose on the development of obesity and diabetes in C57BL/6J and A/J mice. *Metabolism* 44:645–651, 1995

31. Mills E, Kuhn CM, Feinglos MN, Surwit RS: Hypertension in C57BL/6J mouse model of non-insulin dependent diabetes mellitus. *Am J Physiol* 264:R73–R78, 1993

32. Brownlow BS, Petro A, Feinglos MN, Surwit RS: The role of motor activity in diet-induced obesity in C57BL/6J mice. *Physiol Behav* 60:37–41, 1996

33. Surwit RS, Petro AE, Parekh P, Collins S: Low plasma leptin in response to dietary fat in diabetes and obesity-prone mice. *Diabetes* 46:1516–1520, 1997

34. Ring BD, Scully S, Davis CR, Baker MB, Cullen MJ, Pelleymounter MA, Danilenko DM: Systemically and topically administered leptin both accelerate wound healing in diabetic *ob/ob* mice. *Endocrinology* 141:446–449, 2000

35. Sierra-Honigmann MR, Nath AK, Murakami C, Garcia-Cardena G, Papapetropoulos A, Sessa WC, Madge LA, Schechner JS, Schwabb MB, Polverini PJ, Flores-Riveros JR: Biological action of leptin as an angiogenic factor. *Science* 281:1683–1686, 1998

36. Collins S, Daniel KW, Petro AE, Surwit RS: Strain-specific response to β_3 AR agonist treatment of diet-induced obesity in mice. *Endocrinology* 138:405–413, 1997

37. Heymsfield SB, Greenberg AS, Fujioka K, Dixon RM, Kushner R, Hunt T, Lubina JA, Patane J, Self B, Hunt P, McCamish M: Recombinant leptin for weight loss in obese and lean adults: a randomized, controlled, dose-escalation trial. *JAMA* 282:1568–1575, 1999

38. Friedman JM, Halaas JL: Leptin and the regulation of body weight in mammals. *Nature* 395:763–770, 1998