Leptin-Induced Changes in Body Composition in High Fat-Fed Mice[1]

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Female C57BL/6J mice were adapted to 10% or 45% kcal fat diets for 8 weeks. Continuous intraperitoneal infusion of 10 μg of leptin/day from a miniosmotic pump transiently inhibited food intake in low fat-fed but not high fat-fed mice. In contrast, both low and high fat-fed leptin-infused mice were less fat than their phosphate-buffered saline (PBS) controls after 13 days. Leptin infusion inhibited insulin release but did not change glucose clearance in low fat-fed mice during a glucose tolerance test. A single intraperitoneal injection of 30 μg of leptin inhibited 24-hr energy intake and inhibited weight gain in both low and high fat-fed mice. Insulin responsiveness was improved in high fat-fed mice during an insulin sensitivity test due to an exaggerated elevation of circulating insulin concentrations. Thus, leptin infusion reduced adiposity independently of energy intake in high fat-fed mice and improved insulin sensitivity in low fat-fed mice, whereas leptin injections, which produced much greater, but transient, increases in serum leptin concentration, inhibited energy intake in both low and high fat-fed mice. Exp Biol Med 228:24–32, 2003

Key words: leptin infusion; leptin injection; leptin resistance; body composition; IRS protein

Leptin is hypothesized to be a feedback signal in the long-term regulation of energy balance, informing the brain of the size of body fat stores (1). Central administration of exogenous leptin causes a substantial inhibition of food intake and weight loss (2). The inhibition of food intake by leptin is smaller if leptin is administered peripherally than when leptin is injected centrally, but peripheral leptin still promotes weight loss, specifically reducing body fat while protecting lean body mass (3). This tissue-specific response to exogenous leptin is associated with changes in peripheral energy metabolism, which include increased fatty acid oxidation (4) and increased hepatic glucose turnover (5). Some of the metabolic responses may be caused by the direct action of leptin on peripheral tissue. For example, prolonged exposure of adipocytes to leptin *in vitro* leads to the development of insulin resistance (6), whereas leptin inhibits protein breakdown in cultured C_2C_{12} muscle myocytes (7).

Although leptin is thought to act as a negative feedback signal in the regulation of body fat, obese individuals have large fat depots and high circulating concentrations of leptin (8), but normal, or excessive, energy intakes. This dissociation between leptin and appetite has been attributed to "leptin resistance," which may be caused by an inhibition of leptin transport across the blood-brain barrier (9) and/or by an insensitivity of the hypothalamic leptin signaling pathway (10, 11). Several studies have reported that mice fed a high-fat diet develop leptin resistance such that there is no change in food intake when leptin is injected peripherally, but food intake is suppressed when leptin is administered centrally (10, 12).

The leptin receptor that mediates the feeding response has a long intracellular domain and is widely distributed in both central and peripheral tissue, but is present at high concentrations in the hypothalamus (13). In contrast, peripheral tissues contain high concentrations of other leptin receptor subtypes, which have short intracellular domains; *in vitro* studies suggest that they also retain some signaling capability (14). Activation of insulin receptors and some cytokine receptors, such as the interleukin (IL)-4 receptor, causes phosphorylation of insulin receptor substrate (IRS) proteins that allow amplification of the receptor signal and increase the activity of kinases that are downstream in the signaling pathway (15). *In vitro,* activation of leptin receptors transiently promotes tyrosine phosphorylation of IRS proteins in cultured cell lines (16) (17). *In vivo,* bolus intravenous injections of large doses (10 mg/kg) of leptin induce relatively low levels of activation of IRS proteins in peripheral tissues of rats compared with insulin, but stimulate the tyrosine phosphorylation of signal transducer and activator of transcription-3 (STAT3) and mitogen-activated protein kinase (MAPK) as effectively as insulin (18). Therefore, the *in vivo* metabolic responses to leptin may be secondary to leptin activation of proteins in the insulin-signaling cascade.

In this experiment, we tested the effects of leptin infusion or injection on food intake, body composition, glucose

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tolerance, and insulin sensitivity of mice adapted to low- or high-fat diets. At the end of the study, tyrosine phosphorylation of IRS-1 and IRS-2 and the activity of phosphatidylinositol 3-kinase (PI 3-K) associated with either IRS-1 or IRS-2 were measured in different tissues to test whether leptin activated aspects of the insulin signaling cascade *in vivo*. Mice fed low- and high-fat diets were used as it was anticipated that the high fat-fed mice would be leptin resistant and would not show the same responses to leptin as low fat-fed mice. Leptin was administered as a constant intraperitoneal (i.p.) infusion (~0.5 mg/kg/day) from Alzet miniosmotic pumps, producing physiologically relevant changes in circulating leptin concentrations. Additional animals received bolus i.p. injections of larger doses (1.5 mg/kg) of leptin, replicating the method of administration used by other investigators.

Material and Methods

Animals and Diet. Sixty-six female C57BL/6J mice, aged 8 weeks (The Jackson Laboratory, Bar Harbor, ME) were housed individually in cages with grid floors in a room maintained at 78°F with lights on 12 hr/d from 0700 hr. Mice were divided into two weight-matched groups, and one group was fed a low-fat diet containing 10% kcal of fat (Diet D12450B; Research Diets, New Brunswick, NJ) and the other group was fed a high-fat diet containing 45% kcal of fat (Diet D12451; Research Diets). Daily food intakes and body weights were recorded. After 8 weeks of feeding the high-fat diet, each of the dietary groups were divided into three weight-matched groups of 11 mice. All animal procedures were approved by the Institutional Animal Care and Use Committee of the Pennington Biomedical Research Center.

Leptin Sensitivity. The mice were tested for leptin response after 2 days, 2 weeks, and 5 weeks on the experimental diets. On the day of the test, the mice were food deprived from 0700 to 1700 hr. The animals within each dietary treatment were randomly assigned to two groups, one was injected i.p. with 30 μg of leptin (approximately 1.5 mg/kg recombinant murine leptin; R&D Systems, Minneapolis, MN), and the other was injected with an equal volume (0.1 ml) of phosphate-buffered saline (PBS). Food intake was measured 4, 15, and 39 hr after the injection. Body weight was measured before the injection, 15 hr after the injection, and at 24-hr intervals after that.

Insulin Status. To determine whether feeding the high-fat diet for 7 weeks had caused insulin resistance, all of the mice were deprived of food from 0700 to 1500 hr, and a small blood sample was collected by tail-bleeding for measurement of fasting glucose (Sigma Procedure 510; Sigma Chemical Co., St. Louis, MO) and insulin (Rat Insulin RIA; Linco Research Inc., St. Charles, MO).

The Effects of Continuous Leptin Infusion on Insulin Sensitivity and Body Composition of Mice Fed Low- and High-Fat Diets. The experimental design is shown in Figure 1A. Two groups of 11 high fat-fed and two groups of 11 low fat-fed mice were fitted with i.p. Alzet

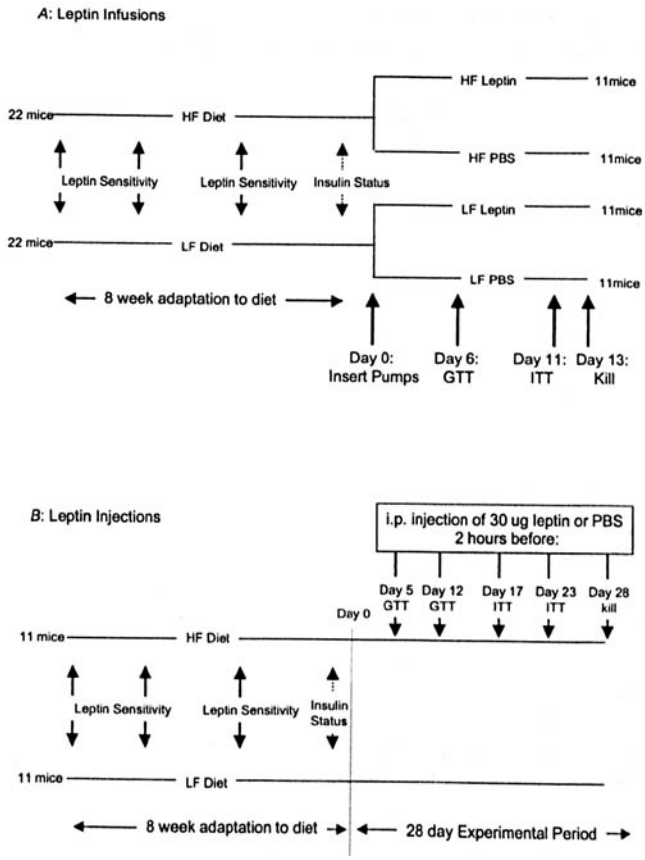

Figure 1. Schematic representation of leptin infusion (A) and leptin injection (B) experimental design.

miniosmotic pumps (Alzet miniosmotic pump model 1002; Durect Corp., Cupertino, CA). One group on each diet was infused with PBS and the other was infused with 10 μg of leptin/day. Six days after pump placement, the infused mice were deprived of food from 0700 to 1200 hr, and an oral glucose tolerance test (OGTT) was performed. Each mouse received 50 mg of glucose by stomach tube, and blood glucose and insulin were measured at 0, 10, 30, and 120 min after gavage. Eleven days after pump placement, the mice were deprived of food once more and were tested for insulin sensitivity (ITT) by measuring blood glucose at 0, 15, 30, 45, and 60 min after an i.p. injection of 0.75 U insulin/kg body weight (HumulinR; Eli Lily, Indianapolis, IN). Small blood samples were collected at the 0- and 15-min time points for measurement of insulin. Blood glucose was measured using a glucometer (Accumet; Boehringer Mannheim, Mannheim, Germany) and if it fell below 20 mg/dl, the mouse was injected i.p. with 50 mg of glucose and was given food. After 13 days of infusion, the mice were deprived of food from 0700 to 1200 hr and were then decapitated. Trunk blood was collected, liver, mesenteric, retroperitoneal, and parametrial fat were weighed, and the gut was cleaned. Parametrial fat, a piece of liver, and the muscle block from the right rear leg were snap frozen. All other tissue was returned to the carcass for determination of body composition, as described previously (19).

The Effect of Single Leptin Injections on Insulin Sensitivity of Mice Fed Low- and High-Fat Diets. The final group of 11 mice on each diet were not infused with leptin, but received i.p. injections of 30 μg of leptin or 100 μl of PBS 2 hr before an OGTT, an ITT, or sacrifice. For both the OGTT and the ITT, each of the injected mice was tested twice, once after a leptin injection and once after a PBS injection. There were at least 5 days between each of the tests. Leptin and PBS were applied in random order. The mice were killed 3 days after the last ITT, which was Day 28 of the experimental period and the mice had been on diet for a total of 12 weeks (see Fig. 1B). One-half of the mice in each dietary treatment group were injected with leptin and one-half were injected with PBS 2 hr before they were sacrificed.

Serum Assays. After glucose and insulin had been measured on the samples collected during the OGTT, the remaining samples were combined for determination of serum leptin concentrations (Mouse Leptin RIA; Linco Research).

Tissue IRS Proteins. The frozen fat, muscle, and liver tissue were homogenized in 25 m*M* Tris, 1.0 m*M* EDTA, and 255 m*M* sucrose, pH 7.4, containing protease and phosphatase inhibitors (1 m*M* phenylmethyl sulfoxide [PMSF], 0.1 mg/ml aprotinin, 10 μg/ml leupeptin, 2.5 m*M* benzamidine hydrochloride, 100 m*M* sodium fluoride, 1 m*M* sodium vanadate, 30 m*M* sodium pyrophosphate, and 1 m*M* sodium molybdate). The homogenate was centrifuged for 30 min at 15,000*g*. Two aliquots from each sample containing 1 mg (fat or muscle) or 2 mg of protein (liver) were diluted to a final volume of 400 μl with homogenizing buffer. Five micrograms of anti-IRS-1 polyclonal antibody (Upstate Technology, Lake Placid, NY) was added to one aliquot and 5 μg of anti-IRS-2 polyclonal antibody (Upstate Technology) was added to the second aliquot. Samples were incubated for 1 hr at 4°C, Protein G suspended on agarose beads (Sigma Chemical Co.) was added to each sample, and after 1 hr, the beads were collected by centrifugation and washed three times with homogenizing buffer. Proteins were separated by gel electrophoresis in a 7% SDS polyacrylamide gel in Tris glycine buffer (25 m*M* Tris, 192 m*M* glycine, and 0.1% SDS, pH 8.3) and transferred to a polyvinylidene difluoride (PVDF) membrane. Phosphotyrosine was detected using monoclonal anti-phosphotyrosine antibody (clone 4G10; Upstate Biotechnology). The blot was stripped and rehybridized for simultaneous detection of PI 3-kinase (Anti-PI 3-kinase p85; Upstate Biotechnology) and IRS-1 or IRS-2. Spot density was determined using a ChemiImager 4000 system (Alpha Innotech, San Leandro, CA) and the ratio of phosphotyrosine or of PI 3-kinase to IRS-1 or IRS-2 was calculated.

Statistical Analysis. Statistically significant differences in single time-point measurements, within either leptin-injected or leptin-infused experiments, were determined by two-way analysis of variance (ANOVA) and *post hoc* Duncan's multiple range test (Statistica; StatSoft, Tulsa, OK) with leptin and diet as independent variables. Differences in repeated measures, including body weight, energy intake, and serum glucose and insulin during the OGTT and the ITT were determined by repeated measures ANOVA using day or time as the repeated measure and diet and leptin as independent variables. *Post hoc* comparisons of treatment means at different time points were tested by *t* test, and significance levels were unadjusted for multiple comparisons (SAS for Windows, release 6.12; SAS Institute, Cary, NC).

Results

Leptin Sensitivity. Leptin responsiveness of the mice during the adaptation period was tested by measuring food intake and body weight following an i.p. injection of 30 μg of leptin or PBS. After 2 days on the high-fat diet, leptin significantly inhibited energy intake ($P < 0.01$) of low fat but not high fat-fed mice 39 hr after the injection (Fig. 2A), but inhibited weight gain ($P < 0.008$) in mice from both dietary groups at both 15 and 39 hr after injection (Fig. 2B). Leptin inhibited energy intake ($P < 0.03$) of mice on both diets 15 hr after injection after 2 weeks of high-fat feeding (Fig. 2C), but had no significant effect on weight gain (Fig. 2D). After 5 weeks of feeding the low- and high-fat diet, leptin inhibited energy intake of low fat-fed mice 39 hr after injection (Fig. 2E; $P < 0.02$), but there was no significant effect of either leptin or of diet on weight gain in the days after injection (Fig. 2F). During the experimental period, the mice were injected on the days that the ITT and OGTT were conducted, therefore, we compared the intakes of the PBS- and leptin-injected mice on these days. In contrast to the pre-experimental period, leptin-injected mice on both diets ate significantly less than PBS controls on all test days (data not shown; leptin: $P < 0.001$; diet: NS; diet × leptin: NS).

Insulin Sensitivity. High fat-fed mice appeared to be insulin resistant after 7 weeks adaptation to the diet, with small but significant elevations of blood glucose (low fat: 123 ± 3 mg/dl; high fat: 138 ± 4 mg/dl; $P < 0.005$) and insulin (low fat: 0.39 ± 0.07 ng/ml; high fat: 0.56 ± 0.06 ng/ml) concentrations. At the end of the 8-week adaptation period, the high fat-fed mice were significantly heavier than low fat-fed mice (low fat: 21.5 ± 0.4 g; high fat: 23.2 ± 0.2 g; $P < 0.001$).

Serum Leptin Concentrations. There were no differences in serum leptin concentrations of low and high fat-fed mice that were treated with PBS during the experimental period (Fig. 3). Infusion of 10 μg of leptin/day caused a 5-fold increase in serum leptin, but injection of 30 μg of leptin increased concentrations 20-fold, 2–3 hr after the injection (Fig. 3).

Food Intake and Body Weight. Diet composition and placement of the pump caused significant changes in energy intake of the mice during the first 4 days of infusion for both dietary groups (Fig. 4; diet: $P < 0.004$; Trt: $P < 0.0001$; Day: $P < 0.0001$; Trt × Day: $P < 0.0001$). In mice

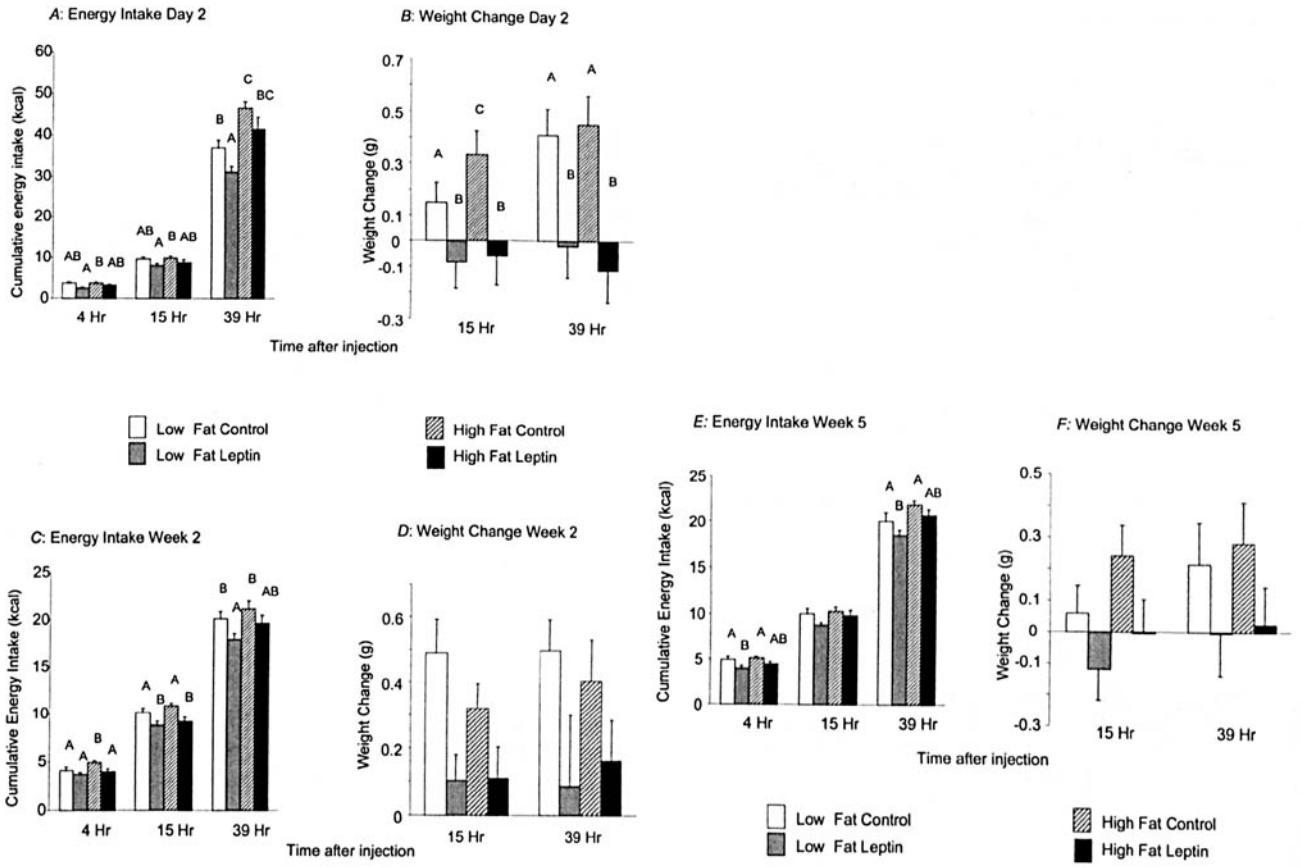

Figure 2. Energy intake and weight change of mice following an i.p. injection of 30 µg of leptin during the 8-week period of adaptation to the low- and high-fat diets. (A and B) Results from mice tested after 2 days of eating the diet; (C and D) Mice that had been eating the diets for 2 weeks; (E and F) Mice that had been eating the diets for 5 weeks. Data are means + SEM for groups of 11 mice fasted for 10 hr before the leptin injection. Values within a given time interval that do not share a common superscript are significantly different at $P < 0.05$, determined by two-way ANOVA and Duncan's Multiple Range Test.

fed a high-fat diet, there was no difference in intakes of PBS-infused and leptin-infused mice. In the low fat-fed animals, leptin-infused mice ate less than PBS-infused animals from Day 3 to Day 6 of infusion (Fig. 4). All of the infused mice lost weight immediately after placement of the Alzet pump (Fig. 5; Diet: $P < 0.001$; Trt: $P < 0.008$; Day: $P < 0.001$; Trt × Day: $P < 0.001$). Leptin-infused, low fat-fed mice weighed significantly less ($P < 0.01$) than noninfused mice from Day 3 of infusion to the end of the experiment. The difference was significant from Day 5 to Day 11 of infusion in high fat-fed mice.

Glucose Tolerance and Insulin Sensitivity. Fasting glucose concentrations were significantly higher in high fat-fed than low fat-fed mice (high fat: 150 ± 11 mg/dl; low fat: 128 ± 9 mg/dl; $P < 0.04$), but leptin had no effect on glucose clearance during the OGTT. Leptin infusion caused a significant ($P < 0.04$) reduction in area under the curve above baseline for insulin release during the OGTT in low fat-fed mice (Fig. 6A), but leptin injection had no effect on the insulin response to glucose (Fig. 6B). Insulin release was greater ($P < 0.01$) in injected than infused mice, irrespective of diet or leptin treatment.

Leptin infusion had no effect on serum glucose concentrations during the ITTs, although the high-fat diet induced insulin resistance (Fig. 7B; Diet: $P < 0.0002$; Leptin: NS; Time: $P < 0.0001$; Diet × Time: $P < 0.01$) and there were significant differences in blood glucose concentrations of high and low fat-fed mice 45 and 60 min after insulin injection. Leptin injection had no effect on blood glucose concentrations (Fig. 7D; Diet: $P < 0.03$; Leptin: NS; Time: $P < 0.0001$; Diet × Time: $P < 0.007$), but insulin sensitivity appeared to be improved in leptin-injected high fat-fed

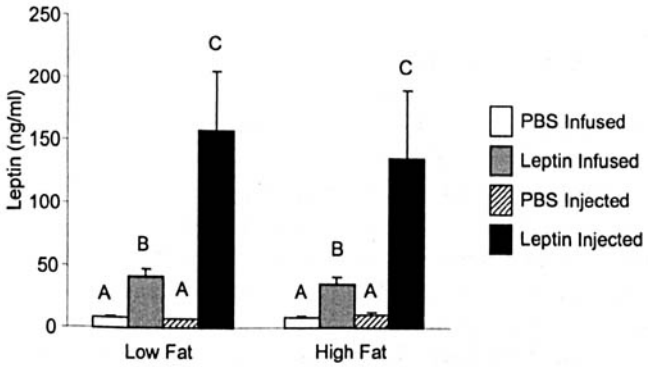

Figure 3. Serum leptin concentrations, measured after 6 days of 10 µg of leptin/d infusion or 2 hr after an i.p. injection of 30 µg of leptin. Values for either low or high fat-fed mice that do not share a common superscript are significantly different at $P < 0.05$, determined by two-way ANOVA and Duncan's Multiple Range Test.

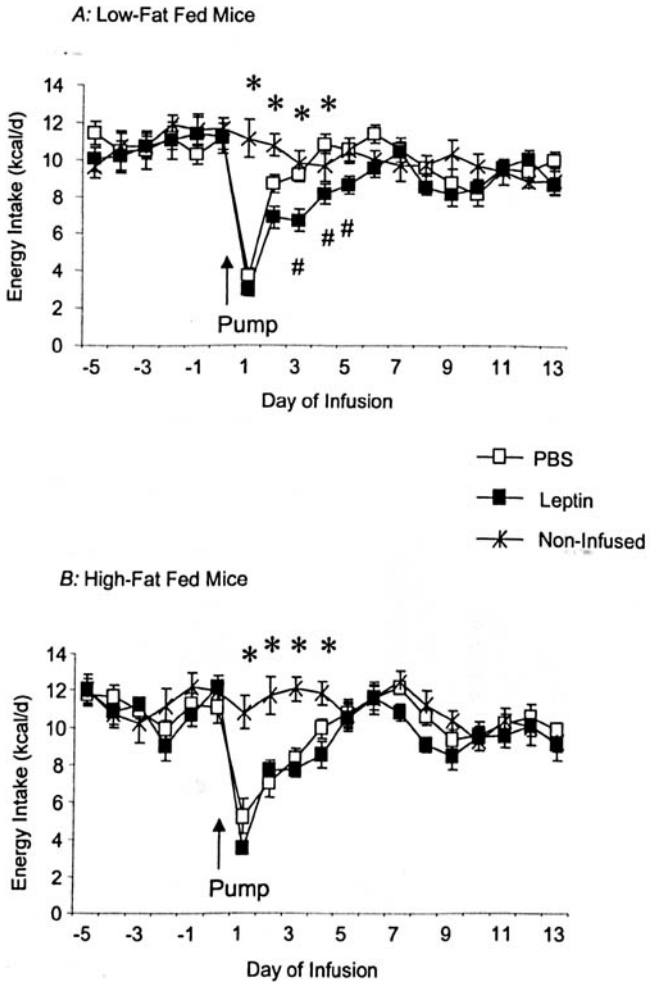

Figure 4. Daily energy intakes of mice fed low-fat (A) or high-fat (B) diets that were infused with PBS or 10 μg of leptin/d or that received i.p. injections of leptin at different times during the experiment (noninfused). Data are means ± SEM or groups of 11 mice. An asterisk indicates a significant ($P < 0.05$) difference between the weights of leptin-infused mice and the noninfused animals, and # indicates a significant ($P < 0.05$) difference between leptin-infused and PBS-infused animals.

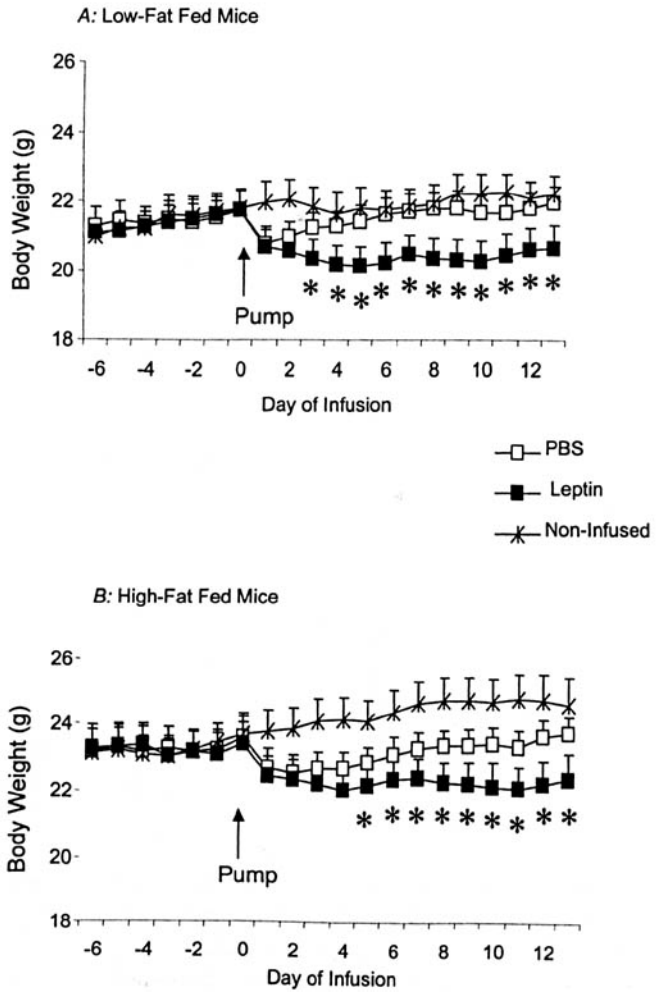

Figure 5. Daily body weights of mice fed a low-fat (A) or high-fat diet (B) and infused with PBS or 10 μg of leptin/d from an i.p. Alzet pump or that received i.p. injections of leptin (noninfused). Data are means + SEM for groups of 11 mice. An asterisk indicates a significant ($P < 0.05$) difference between the weights of leptin-infused mice and the noninfused animals.

mice. This may have been because high fat-fed, leptin-injected mice had elevated serum insulin concentrations 15 min after leptin injection (Fig. 7C; Diet: NS; Leptin: $P < 0.004$; Diet × Leptin: $P < 0.04$).

Body Composition. Injection of leptin 2 hr before sacrifice had no effect on body composition (data not shown). Leptin infusion caused a significant reduction in parametrial and retroperitoneal fat in both the high and low fat-fed animals and in mesenteric fat from the high fat-fed mice (see Table I). Carcass weight, total body fat content, and carcass protein were also reduced in leptin-infused animals on both diets. The loss of fat was greater than the loss of lean tissue (Table I).

IRS Proteins. None of the data on activation of IRS proteins is shown because leptin had little effect on phosphorylation of IRS1 or IRS2 or on the association of PI3-kinase with these signaling proteins compared with what would be expected with activation of insulin receptors. The high-fat diet increased the tyrosine phosphorylation of IRS-1 ($P < 0.03$) in adipose tissue by approximately 50%, and leptin injection increased tyrosine phosphorylation of IRS-1 approximately 2-fold ($P < 0.04$) in fat from high fat-fed mice. The high-fat diet reduced tyrosine phosphorylation of IRS-1 in muscle of leptin-injected mice by 50% ($P < 0.03$). In the liver, leptin infusions caused a 50% reduction ($P < 0.07$) in PI 3-kinase activity associated with IRS-1, whereas leptin injection doubled tyrosine phosphorylation of IRS-2 ($P < 0.04$).

Discussion

In this experiment, we examined the effects of acute injections and of continuous infusions of leptin on whole body glucose clearance, insulin sensitivity, and noninsulin-stimulated levels of tyrosine phosphorylation and PI 3-kinase activity associated with IRS-1 and IRS-2 in insulin-sensitive tissues. Leptin infusions appeared to have insulin-

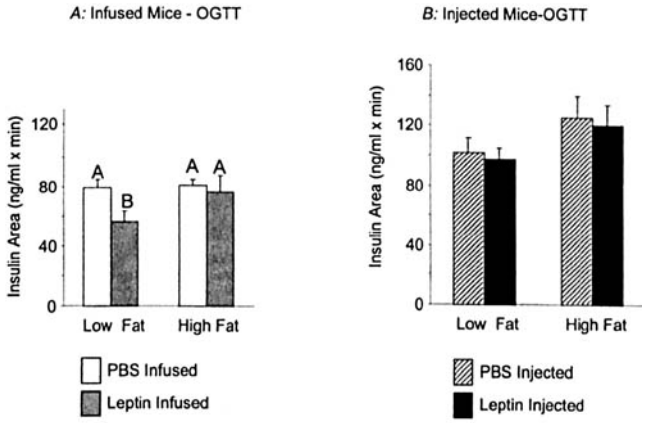

Figure 6. Area under the curve above baseline for insulin during the glucose tolerance test performed on Day 6 of infusion. (A) Insulin for infused-mice; (B) Insulin for injected mice. Data are means + SEM for groups of 11 mice. Superscripts indicate that insulin release was significantly ($P < 0.05$) inhibited in low fat-fed mice infused with leptin.

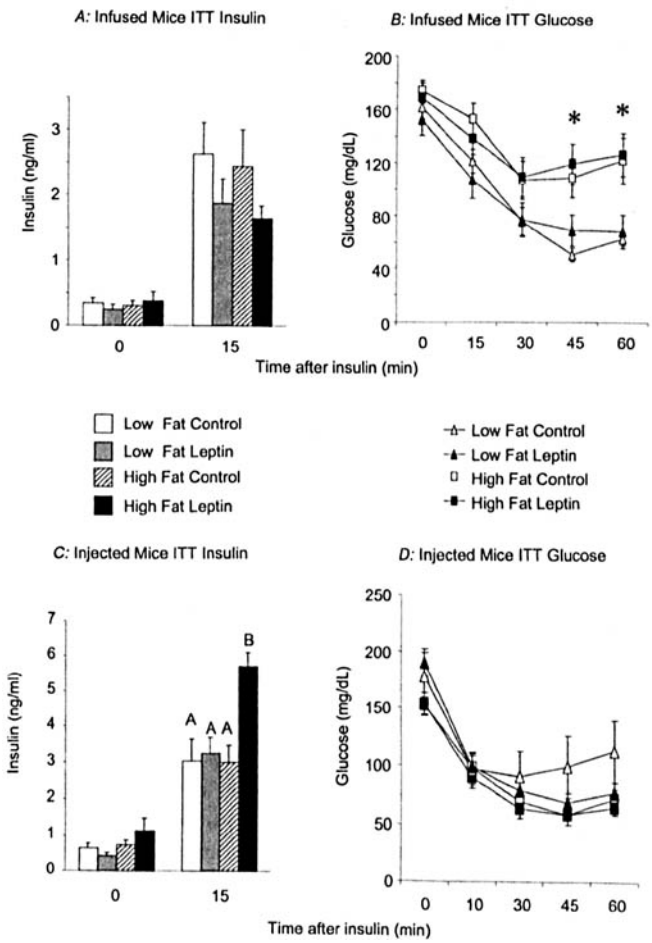

Figure 7. Insulin and glucose measured before and after an i.p. injection of 0.75 U/kg insulin in the ITT test. (A) Insulin for infused mice; (B) Glucose for infused mice; (C) Insulin for injected mice; and (D) Glucose for injected mice. Data are means ± SEM for groups of 11 mice. An asterisk indicates a significant ($P < 0.05$) difference between high and low fat-fed mice. Superscripts on the values for insulin in leptin injected mice indicate significant ($P < 0.05$) differences between the groups 15 min after insulin injection. These mice had been injected with leptin 2 hr before the start of the test.

like effects in low fat-fed mice, reducing the amount of insulin released in response to a glucose challenge while maintaining the same rate of glucose clearance as in PBS-infused mice. These changes in insulin requirement were not associated with any changes in basal levels of tyrosine phosphorylation or of PI 3-kinase activity associated with IRS-1 or IRS-2 in the insulin-responsive tissues. All of the measurements made on leptin-injected mice were initiated 2 hr after the injection, when serum leptin concentrations were still elevated 20-fold. Leptin injections did not change insulin release during the glucose tolerance test but appeared to increase insulin sensitivity by either inhibiting insulin clearance or by increasing insulin absorbance after the leptin injection. The difference in serum insulin concentrations between leptin-injected low fat-fed and high fat-fed mice could not be attributed to the difference in size of the mice as the weight difference was approximately 2 g (<10% of body weight), whereas insulin concentrations were more than doubled.

It has been shown that continuous infusion of leptin into fasted rats lowers circulating concentrations of both glucose and insulin and that an acute infusion of leptin improves insulin sensitivity in rats with clamped glucose concentrations (20), implying that leptin has insulin-like activity. Results from *in vitro* studies suggest that leptin may change insulin sensitivity of peripheral tissues by modifying the activation of proteins in the insulin signaling cascade, but the effects vary between cell lines (21). Additionally, leptin has been shown to stimulate glucose transport and glycogen synthesis in C_2C_{12} myotubes (22) by a pathway that is dependent upon phosphorylation of JAK2, IRS-2, and PI 3-kinase (17). *In vivo*, a large (1 mg/kg × 3 min) acute intravenous infusion of leptin stimulates adipose tissue IRS-1-associated PI 3-kinase activity and liver IRS-2-associated PI 3-kinase in rats. In addition, leptin increases the phosphorylation of STAT proteins in fat, muscle, and liver and increases MAPK phosphorylation in adipose tissue (18), a response that has been associated with proliferation of other cell types *in vitro* (23). Therefore, if leptin is capable of inhibiting or promoting phosphorylation of specific sites in the insulin signaling cascade, there is the potential to accentuate some aspects of insulin action while inhibiting others.

In this experiment, leptin infusion had no effect on basal activation of IRS-1 or IRS-2 in the insulin-responsive tissues in leptin-infused animals. Thus, the improved insulin sensitivity observed in the OGTT of low fat-fed, leptin-infused mice was due to either a direct effect of leptin on tissue glucose transport or to leptin amplifying postreceptor events initiated by insulin stimulation. In contrast, leptin injection increased tyrosine phosphorylation of IRS-1 in adipose tissue and of IRS-2 in liver 2-fold. These responses are similar to those described for rats that received intravenous leptin infusions (18), and are much smaller than would be produced by insulin (18). The small changes in basal

Table I. Body Composition and Fat Pad Weights of Infused Mice

	Low-fat		High-fat		Statistical Summary
	PBS	Leptin	PBS	Leptin	
Organ Weights (mg)					
Liver	893 ± 26	882 ± 49	895 ± 30	849 ± 35	
Mesenteric Fat	184 ± 19^{AB}	124 ± 29^{A}	200 ± 24^{B}	136 ± 17^{AB}	D: NS, Lep: P < 0.01, DxL: NS
Retroperitoneal Fat	77 ± 8^{A}	40 ± 14^{B}	141 ± 15^{C}	76 ± 14^{A}	D: P < 0.001, Lep: P < 0.001, DxL: NS
Parametrial Fat	429 ± 42^{A}	225 ± 3^{B}	724 ± 60^{C}	464 ± 85^{A}	D: P < 0.001, Lep: P < 0.001, DxL: NS
Carcass Composition (g)					
Weight	18.5 ± 0.4^{AB}	17.5 ± 0.5^{B}	20.0 ± 0.4^{C}	19.0 ± 0.6^{A}	D: P < 0.05, Lep: P < 0.04, DxL: NS
Fat	2.8 ± 0.3^{AC}	1.2 ± 0.2^{B}	3.3 ± 0.2^{A}	2.5 ± 0.4^{C}	D: P < 0.001, Lep: P < 0.002, DxL: NS
Water	10.8 ± 0.2	11.0 ± 0.3	11.3 ± 0.2	11.3 ± 0.2	
Protein	4.3 ± 0.1	4.1 ± 0.1	4.5 ± 0.1	4.3 ± 0.1	D: P < 0.04, Lep: P < 0.04, DxL: NS
Ash	0.97 ± 0.03	0.91 ± 0.05	0.95 ± 0.04	0.91 ± 0.04	

Note. Data are means ± SEM for groups of 11 mice infused i.p. with either PBS or leptin for 14 days and fed either low- or high-fat diet, for 8 weeks before the start of infusion. Values for carcass fat content that do not share a common superscript are significantly different at $P < 0.05$, determined by two-way ANOVA and *post hoc* Duncan's Multiple Range Test. D = Diet, Lep = Leptin.

levels of IRS phosphorylation were not associated with an improved glucose tolerance of the mice, but it is possible that leptin induced other insulin-like activity, such as cell proliferation. In mice fed the high-fat diet, there was a 50% inhibition of basal levels of IRS-1 phosphorylation in muscle, which may have contributed to the mild state of insulin resistance in these animals.

Continuous infusion of leptin into low and high fat-fed mice for 13 days caused a significant reduction in the body fat content of both dietary groups even though food intake was inhibited only in the low fat-fed animals. Others have reported that feeding a high-fat diet induces resistance to peripherally administered leptin in rodents. In this experiment, we found varied responses to leptin injection in mice fed a high-fat diet for different periods of time. After 5 weeks on the high-fat diet, the mice appeared to be "leptin resistant," but after 9 weeks, food intake was significantly inhibited by leptin. The inhibition of feeding in response to leptin injection was unexpected as we used the same strain of mice and the same diet as others who have reported leptin resistance (10, 24). One difference between the experiments was that the other investigators used male mice, whereas we used females based on previous observations that they are more responsive to leptin than males (25; R.B.S. Harris, unpublished observations). If there is a gender difference in leptin responsiveness, then it may be related to estrogen producing a cyclic variation in hypothalamic leptin receptor expression (26). Alternatively, Vasselli *et al.* (27) reported that high fat-fed rats only become leptin resistant once they are obese. The high fat-fed mice in this experiment were not significantly fatter than the low fat-fed animals and this may have allowed retention of leptin sensitivity.

Leptin infusion increased circulating concentrations of the protein by approximately 5-fold, which is well within the range found in obese mice. The continuous elevation of leptin caused only a transient inhibition of energy intake in the low fat-fed mice and did not change energy intake of the high fat-fed animals, suggesting that these animals had developed a central "resistance" to small increases in circulating leptin concentrations. Despite this difference in the feeding response of the two dietary groups, all of the mice lost body fat during the 12 days of infusion, implying that leptin stimulated energy expenditure even in the high fat-fed animals. The increased expenditure has been attributed to sympathetic activation of uncoupling proteins (UCPs) (28) in a response that is mediated by central leptin receptors (29). High-fat diets suppress sympathetic activity (30), but the loss of fat in leptin-infused mice in this experiment suggests that leptin-induced stimulation of expenditure remained intact. It remains to be determined whether the increase in expenditure is entirely dependent upon activation of UCPs or whether other futile cycles contribute to the reduced efficiency of energy utilization.

The inhibition of food intake by leptin is mediated by the long-form leptin receptor in the hypothalamus (13). Because the high fat-fed mice did not reduce their food intake during leptin infusion, it may be assumed that leptin either did not reach these receptors or that the receptors were not responsive to the 5-fold increase in leptin. However, the increased energy expenditure in these mice must have been due to activation of leptin receptors that are independent of those that inhibit food intake. We did not measure the expression of either long- or short-form receptors as others have been unable to find a significant difference in the levels of expression in high fat-fed mice (10) or rats (11), although the amount of both long- and short-form leptin receptor protein in the hypothalamus of high fat-fed rats is reduced compared with that in rats fed a low-fat diet (11). It has not been determined whether a high-fat diet downregulates leptin receptor protein in peripheral tissues.

As expected, the composition of weight loss in both the high and low fat-fed mice was primarily adipose tissue with a small reduction in lean body mass. These changes are consistent with reports that leptin promotes adipose tissue lipolysis (31) and fatty acid oxidation in multiple tissues (32), while inhibiting protein catabolism (7). Although weight loss in mice maintaining a normal food intake re-

quires energy expenditure to be increased, the specific loss of fat from leptin-infused mice was not necessarily the simple result of negative energy balance. If leptin stimulates the sympathetic nervous system, there will be an associated increase in lipolysis and fatty acid oxidation. However, it has also been reported that leptin depletes denervated fat depots (33) and that adipocytes isolated from high fat-fed rats resistant to the feeding effects of peripheral leptin retain their sensitivity to the direct stimulation of lipolysis by leptin *in vitro* (34) via the long-form leptin receptor (31). Thus, although the hypothalamic receptor in high fat-fed mice or rats does not respond to peripheral leptin administration, it appears that long-form receptors in peripheral tissue are still responsive to the protein. Alternatively, some of the reduction in body fat mass of the mice may have been caused by an inhibition of insulin-stimulated lipogenesis. Leptin does not modify adipocyte glucose metabolism in basal conditions, but both *in vitro* (35) and *in vivo* (36) studies demonstrate that prolonged exposure to leptin inhibits insulin-stimulated glucose utilization by adipocytes. This inhibition of insulin action is unlikely to be a direct effect of leptin on the cells because acute exposure of isolated adipocytes to leptin augments insulin-stimulated lipid synthesis (36).

In summary, feeding mice a high-fat diet may reduce central sensitivity to leptin, as there was no change in food intake in response to infusions that increased serum leptin concentrations approximately 5-fold, although intake was inhibited by bolus injections that produced much greater elevations in serum leptin. Despite the lack of change in food intake, body fat content was reduced by leptin infusions in high fat-fed mice. This loss of fat may have resulted from both a stimulation of energy expenditure by leptin and by a direct effect of leptin on adipose tissue lipid metabolism. Leptin infusions did not change insulin sensitivity of the mice, but augmented insulin action when the mice were given a glucose challenge. In contrast, leptin injections increased glucose clearance in high fat-fed mice by maintaining insulin at an elevated level. Leptin injections also produced small increases in the activation of IRS proteins in fat and liver tissue that may be related to other actions of insulin and leptin, such as angiogenesis.

1. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature **372:**425–432, 1994.
2. 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.
3. Harris RB, Zhou J, Redmann SM Jr, Smagin GN, Smith SR, Rodgers E, Zachwieja JJ. A leptin dose-response study in obese (*ob/ob*) and lean (+/?) mice. Endocrinology **139:**8–19, 1998.
4. Unger RH, Zhou YT, Orci L. Regulation of fatty acid homeostasis in cells: novel role of leptin. Proc Natl Acad Sci U S A **96:**2327–2332, 1999.
5. Kamohara S, Burcelin R, Halaas JL, Friedman JM, Charron MJ. Acute stimulation of glucose metabolism in mice by leptin treatment. Nature **389:**374–377, 1997.
6. Mick G, Vanderbloomer T, Fu CL, McCormick K. Leptin does not affect adipocyte glucose metabolism: studies in fresh and cultured adipocytes. Metabolism **47:**1360–1365, 1998.
7. Ramsay T. Leptin inhibits protein breakdown in muscle cells. FASEB J **12:**A865, 1998.
8. Considine RV, Sinha MK, Heiman ML, Kriauciunas 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.
9. Banks WA, Clever CM, Farrell CL. Partial saturation and regional variation in the blood-to-brain transport of leptin in normal weight mice. Am J Physiol **278:**E1158–E1165, 2000.
10. El-Haschimi K, Pierroz DD, Hileman SM, Bjorbaek C, Flier JS. Two defects contribute to hypothalamic leptin resistance in mice with diet-induced obesity. J Clin Invest **105:**1827–1832, 2000.
11. Madiehe AM, Schaffhauser AO, Braymer DH, Bray GA, York DA. Differential expression of leptin receptor in high- and low-fat-fed Osborne-Mendel and S5B/Pl rats. Obesity Res **8:**467–474, 2000.
12. Van Heek M, Compton DS, France CF, Tedesco RP, Fawzi AB, Graziano MP, Sybertz EJ, Strader CD, Davis HR Jr. Diet-induced obese mice develop peripheral, but not central, resistance to leptin. J Clin Invest **99:**385–390, 1997.
13. Tartaglia LA. The leptin receptor. J Biol Chem **272:**6093–6096, 1997.
14. Bjorbaek C, Uotani S, da Silva B, Flier JS. Divergent signaling capacities of the long and short isoforms of the leptin receptor. J Biol Chem **272:**32686–32695, 1997.
15. White MF. The IRS-signalling system in insulin and cytokine action. Phil Trans R Soc Lond B Biol Sci **351:**181–189, 1996.
16. Szanto I, Kahn CR. Selective interaction between leptin and insulin signaling pathways in a hepatic cell line. Proc Natl Acad Sci U S A **97:**2355–2360, 2000.
17. Kellerer M, Koch M, Metzinger E, Mushack J, Capp E, Haring HU. Leptin activates PI-3 kinase in C2C12 myotubes via janus kinase-2 (JAK-2) and insulin receptor substrate-2 (IRS-2) dependent pathways. Diabetologia **40:**1358–1362, 1997.
18. Kim YB, Uotani S, Pierroz DD, Flier JS, Kahn BB. In vivo administration of leptin activates signal transduction directly in insulin-sensitive tissues: overlapping but distinct pathways from insulin. Endocrinology **141:**2328–2339, 2000.
19. Harris RB. Growth measurements in Sprague-Dawley rats fed diets of very low fat concentration. J Nutr **121:**1075–1080, 1991.
20. Sivitz WI, Walsh SA, Morgan DA, Thomas MJ, Haynes WG. Effects of leptin on insulin sensitivity in normal rats. Endocrinology **138:**3395–3401, 1997.
21. Cohen B, Novick D, Rubinstein M. Modulation of insulin activities by leptin. Science **274:**1185–1188, 1996.
22. Berti L, Kellerer M, Capp E, Haring HU. Leptin stimulates glucose transport and glycogen synthesis in C2C12 myotubes: evidence for a P13-kinase mediated effect. Diabetologia **40:**606–609, 1997.
23. Takahashi Y, Okimura Y, Mizuno I, Iida K, Takahashi T, Kaji H, Abe H, Chihara K. Leptin induces mitogen-activated protein kinase-dependent proliferation of C3H10T1/2 cells. J Biol Chem **272:**12897–12900, 1997.
24. Van Heek M, Mullins DE, Wirth MA, Graziano MP, Fawzi AB, Compton DS, France CF, Hoos LM, Casale RL, Sybertz EJ, Strader CD, Davis HR Jr. The relationship of tissue localization, distribution and turnover to feeding after intraperitoneal 125I-leptin administration to *ob/ob* and *db/db* mice. Horm Metab Res **28:**653–658, 1996.
25. Sarmiento U, Benson B, Kaufman S, Ross L, Qi M, Scully S, DiPalma C. Morphologic and molecular changes induced by recombinant human leptin in the white and brown adipose tissues of C57BL/6 mice. Lab Invest **77:**243–256, 1997.
26. Bennett PA, Lindell K, Wilson C, Carlsson LM, Carlsson B, Robinson IC. Cyclical variations in the abundance of leptin receptors, but not in circulating leptin, correlate with NPY expression during the oestrous cycle. Neuroendocrinology **69:**417–423, 1999.
27. Vasselli J, Koczka C, Singh K, Boozer C, Maggio C. Expression of

leptin resistance: role of diet versus increased adiposity. Obesity Res 8(Suppl 1):14S, 2000.
28. Scarpace PJ, Matheny M, Pollock BH, Tumer N. Leptin increases uncoupling protein expression and energy expenditure. Am J Physiol 273:E226–E230, 1997.
29. Cusin I, Zakrzewska KE, Boss O, Muzzin P, Giacobino JP, Ricquier D, Jeanrenaud B, Rohner-Jeanrenaud F. Chronic central leptin infusion enhances insulin-stimulated glucose metabolism and favors the expression of uncoupling proteins. Diabetes 47:1014–1019, 1998.
30. Lu H, Duanmu Z, Houck C, Jen KL, Buison A, Dunbar JC. Obesity due to high fat diet decreases the sympathetic nervous and cardiovascular responses to intracerebroventricular leptin in rats. Brain Res Bull 47:331–335, 1998.
31. Fruhbeck G, Aguado M, Martinez JA. In vitro lipolytic effect of leptin on mouse adipocytes: evidence for a possible autocrine/paracrine role of leptin. Biochem Biophys Res Commun 240:590–594, 1997.
32. Shimabukuro M, Koyama K, Chen G, Wang MY, Trieu F, Lee Y, Newgard CB, Unger RH. Direct antidiabetic effect of leptin through triglyceride depletion of tissues. Proc Natl Acad Sci U S A 94:4637–4641, 1997.
33. Wang ZW, Zhou YT, Lee Y, Higa M, Kalra SP, Unger RH. Hyperleptinemia depletes fat from denervated fat tissue. Biochem Biophys Res Commun 260:653–657, 1999.
34. Johnson J, Ng K, Albu J, Vasselli J. High fat-fed rats resistant to the feeding inhibitory effect of leptin are responsive to its lipolytic effect in adipose tissue. Obesity Res 8(Suppl 1):22S, 2000.
35. Muller G, Ertl J, Gerl M, Preibisch G. Leptin impairs metabolic actions of insulin in isolated rat adipocytes. J Biol Chem 272:10585–10593, 1997.
36. Harris RB. Acute and chronic effects of leptin on glucose utilization in lean mice. Biochem Biophys Res Commun 245:502–509, 1998.