Intracerebroventricular Administration of Leptin Markedly Enhances Insulin Sensitivity and Systemic Glucose Utilization in Conscious Rats

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This study examines the acute, subacute (overnight), and chronic (7-day) effects of intracerebroventricular (ICV) administration of r-metMuLeptin on insulin sensitivity and systemic glucose turnover in conscious unrestrained rats (body weight, 250 to 300 g). Under postabsorptive conditions, acute ICV leptin ([AL] 10 μg bolus) did not affect tracer (3-^{3}H-glucose)-determined glucose production (GP) and utilization (GU) rates during the 2-hour hyperinsulinemic (2 mU · kg^{-1} · min^{-1}) euglycemic clamp. Chronic ICV leptin ([CL] 10 μg/d for 7 days) administered by osmotic pumps markedly reduced the daily food consumption ($P < .05$), body weight ($P < .05$), and postabsorptive basal plasma glucose level ($P < .01$). During the glucose clamp, GP was markedly suppressed (55%) with CL ($P < .001$ *v* vehicle and pair-fed control groups). The insulin-induced increment in GU was significantly greater with CL (23.3 ± 1.8 mg^{-1} · kg^{-1} · min^{-1}) than with vehicle (16.9 ± 0.2) and pair-feeding (17.1 ± 0.6, both $P < .001$). Subacute ICV leptin ([SL] 10 μg bolus) moderately but insignificantly decreased overnight food consumption (−18%) and body weight (−2.5 ± 1.5 g). The glucose infusion rate during the final 60 minutes of the glucose clamp was 43% greater than for the vehicle group ($P < .0001$). SL also significantly increased GU ($P < .005$) and suppressed GP ($P < .05$) during the glucose clamp. Thus, we conclude that ICV administered leptin has strong actions on the central nervous system that result in significant increases in insulin sensitivity and systemic GU, and these effects are achieved as early as overnight after leptin administration.

SINCE THE DISCOVERY of the mouse obese (ob) gene and its human homolog,[1] the cumulative evidence suggests that leptin, the protein product of the ob gene, plays an important role in the regulation of food intake and body weight.[2-4] The genetically defective leptin production in ob/ob mice and the genetically altered leptin receptors in db/db mice and Zucker fa/fa rats all result in obese phenotypes. However, the effects of leptin in adiposity reduction are not entirely achieved through restriction of food intake, as demonstrated in pair-fed control studies.[5] Many studies support the concept that leptin plays an important role in the regulation of energy metabolism.[2,6-10] Leptin treatment was shown to significantly increase energy expenditure in ob/ob mice compared with vehicle control animals.[2] In normal lean mice, leptin treatment prevented the significant reduction in energy expenditure due to food restriction as observed in pair-fed control animals.[10] In obese humans, the circulating leptin concentration was inversely correlated with the resting energy expenditure, respiratory quotient, and carbohydrate oxidation rate.[11] Elevated leptin levels in obese humans are suggestive of a reduced sensitivity to endogenous leptin,[12,13] and thus the reduced energy expenditure in human obesity may be the consequence of a relative leptin insufficiency.

A well-established metabolic feature of obesity in both humans and animal models is insulin resistance.[14,15] Most of the non–insulin-dependent diabetic (NIDDM) individuals who are insulin-resistant and relatively insulin-deficient are overweight.[15] A central issue in the treatment of obesity and NIDDM is therefore the improvement of insulin sensitivity. The aim of this study is to determine if centrally administered leptin affects systemic insulin sensitivity and whole-body glucose utilization in vivo.

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MATERIALS AND METHODS

Animals and Surgical Procedures

Healthy female Sprague-Dawley rats (body weight, 250 to 300 g; Harlan Sprague-Dawley, San Diego, CA) were housed with a 12-hour light-dark cycle and fed regular rat chow, and the daily consumption was recorded. All animals in the acute and subacute intracerebroventricular (ICV) leptin studies were allowed food ad libitum. In the chronic ICV leptin study, the amount of food consumed by leptin-treated animals was recorded daily, and pair-fed animals received the daily amount equivalent to the daily average of the leptin-treated animals. Two to 3 weeks before the experiments, the rats received stereotaxic implantation of stainless steel cannulae into a lateral ventricle according to stereotaxic coordinates[16] under general anesthesia (3% to 4% isofluorane in oxygen). Five to 6 days before the experiments, the animals received chronic cannulation of the left carotid artery and right jugular vein using PE-50 intramedic tubing fitted with a short segment of Medical Silicone tubing (.020/.037 in ID/OD; Baxter, Hayward, CA) for flexible, noninjurious insertion into the vessels.[17] The cannulae were tunneled subcutaneously and exteriorized from the back of the neck and then filled with a mixed solution containing 60% polyvinylpyrrolidone (Sigma Chemical, St Louis, MO) and 1,000 U/mL heparin in normal saline.[17] The cannulae can be maintained patent for as long as 7 to 8 days without the need for reflushing and reheparinization. To verify the technical integrity of the ICV injection and experiments, positive control experiments were performed in two animals using ICV injection of carbachol, an acetylcholine analog, which was shown to induce a general state of stress.[18,19] In our positive control experiments, acute ICV injection of carbachol (5 μg/2 μL) resulted in dramatic increases in plasma glucose levels and tracer-determined whole-body glucose turnover (unpublished data). All animal procedures were approved by the Laboratory Animal Resources Committee at Amgen.

Acute ICV Leptin Study

This study examines the effect of acute ICV leptin at basal insulin levels. There were six rats each in both the leptin and vehicle groups. The animals were fasted for 5 to 6 hours before the experiments. The contents of the vascular cannulae were aspirated, and the cannulae were extended with PE-50 plastic tubing and flushed with fresh heparinized

(10 U/mL) saline. The jugular vein cannula was used for infusion of tracer, insulin, and glucose via serial "Y" needle connectors. A primed constant infusion (0.36 μCi, 0.07 μCi/min) of 3-[^{3}H]-glucose (NEN, Boston, MA) was administered to determine systemic glucose appearance (Ra) and disappearance (Rd) rates. The experiments consisted of a tracer equilibration period (from −120 to −60 minutes), a basal sampling period (from −60 to 0 minutes), and an ICV leptin/vehicle study period (from 0 to 120 minutes). Constant insulin infusion (2 mU · kg^{-1} · min^{-1}) was begun at 0 minutes, when r-metMuLeptin (10 μg/2 μL) or Dulbecco's phosphated buffer ([DPB] 2 μL) were administered ICV, and lasted until the end of the experiment. An exogenous glucose infusion (30%) was administered at variable rates according to instant plasma glucose measurements, to maintain basal plasma glucose levels. Arterial samples were taken at timed intervals from the carotid cannula and centrifuged using a microfuge. Plasma samples were collected in ice-chilled Eppendorf microtubes containing sodium fluoride (for glucose tracer measurements) or EGTA/aprotinin (for hormones and metabolites) and stored at −70°C until assay.[17] After removal of the plasma from the blood samples, the packed blood cells were resuspended in heparinized saline and reinfused after each blood sampling, to prevent volume depletion and anemia as previously described.[17]

Subacute ICV Leptin Study

This study examines the effects of subacute (overnight) ICV leptin in both a leptin group (n = 6) and a DPB vehicle group (n = 6). Animals received ICV r-metMuLeptin (10 μg/2 μL) or vehicle injections at 4:00 to 5:00 PM on the day before the experiment. The body weight and food amount were recorded. At 7:00 AM on the day of the experiments, the animals were weighed and overnight food consumption was determined. The experiments were started after a 5- to 6-hour fast and consisted of a tracer equilibration period (−120 to −60 minutes), a basal sampling period (−60 to 0 minutes), and a hyperinsulinemic (2 mU · kg^{-1} · min^{-1})-euglycemic clamp period (0 to 180 minutes). The rest of the experimental procedures were similar to the acute study already described.

Chronic ICV Leptin Study

The chronic (7-day) ICV leptin study used three groups: leptin (n = 6), vehicle control (n = 5), and pair-fed vehicle (n = 7). In this study, r-metMuLeptin was infused by subcutaneously implanted osmotic pumps (model 2002; Alzet Pharmaceuticals, Palo Alto, CA) at a rate of 10 μg/rat/d. Both the vehicle and pair-fed groups received DPB 12 μL/d. Body weight and food consumption were recorded daily, and pair-fed rats were fed a daily ration equivalent to the average daily consumption of the leptin group. After a 5- to 6-hour fast, the experiments were initiated with a tracer equilibration period (−120 to −60 minutes). This was followed by a basal sampling period (−60 to 0 minutes) and a hyperinsulinemic (2 mU · kg^{-1} · min^{-1}) euglycemic clamp period (0 to 180 minutes). The rest of the experimental procedures were the same as for the acute and subacute studies already described.

Laboratory Methods

Plasma glucose concentrations were measured by a glucose oxidase method using a Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA). Plasma glucose specific activity was derived from the plasma glucose concentration and [3-^{3}H]glucose radioactivity determined by liquid scintillation spectrometry on a Beckman LS60001C counter. The samples were read after deproteinization with $Ba(OH)_2$ and $ZnSO_4$ and overnight evaporation of the supernatant at 70°C.[20] The glucose Ra and Rd in the circulation were calculated with an equation for non–steady-state turnover using a one-compartment model[21] that has been validated.[22] Raw data for the glucose concentration and specific activity were smoothed according to the optimal-segments method.[23] Tracer-derived Rd represents systemic glucose utilization (GU). During the basal period, hepatic glucose production (GP) was equated to the glucose Ra. During the hyperinsulinemic-euglycemic clamp, GP was calculated as the difference between the Ra and rate of exogenous glucose infusion. To avoid rapid changes in glucose specific activity due to exogenous glucose infusion, the tracer-determined glucose Ra and Rd during the glucose clamps were only measured in the last 30 minutes (90 to 120 minutes) for the acute leptin study and in the last 60 minutes (120 to 180 minutes) for the subacute and chronic leptin studies when the adjustment in glucose infusion rates became minimal for at least 30 minutes. Analyses of the biochemical parameters cholesterol, triglyceride, corticosterone, β-hydroxybutyrate, free fatty acids, lactate, and glycerol were performed spectrophotometrically on a Hitachi 717 Clinical Chemistry Autoanalyzer (Boehringer Mannheim, Indianapolis, IN). Insulin levels were measured with a double-antibody, ruthenium-electrochemiluminescent method. The leptin plasma concentration was measured by an enzyme-linked immunoassay.[24]

Statistical Analysis

All data are expressed as the mean ± SEM. Two-way ANOVA or an unpaired *T* test were used to determine statistical differences among the three groups or between two groups, respectively. Significance is assumed at *P* less than .05.

RESULTS

Acute ICV Leptin

Basal plasma glucose levels were similar between the acute leptin ([AL] 125.7 ± 3.1 mg/dL) and acute vehicle ([AV] 127.9 ± 1.5 mg/dL) groups. After acute ICV leptin injection and during the hyperinsulinemic-euglycemic clamp, plasma glucose levels were maintained at similar levels between the two groups (AL *v* AV, 117.4 ± 3.1 *v* 119.2 ± 2.0 mg/dL, *P* = NS; Fig 1). Glucose production rates were 9.3 ± 0.3 and 9.5 ± 0.4 mg · kg^{-1} · min^{-1} (AL *v* AV, *P* = NS) in the basal

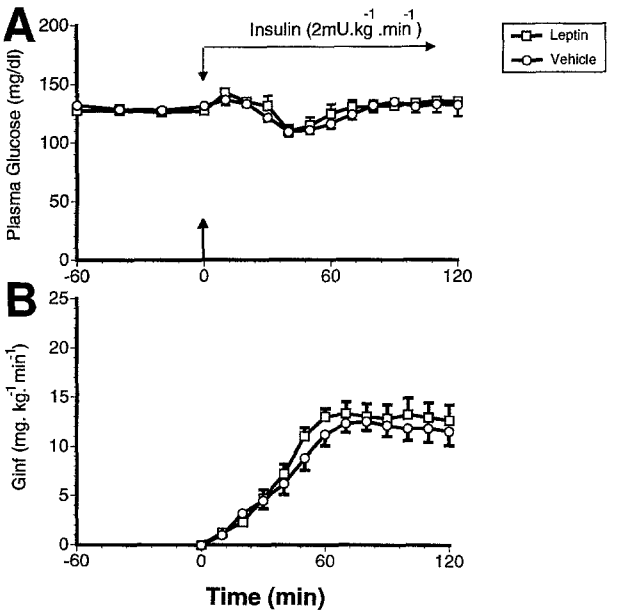

Fig 1. (A) Plasma glucose levels in acute ICV leptin (10 μg, 0 minute, n = 6) and vehicle (n = 6) treatment in rats during basal (−60-0 minutes) and glucose clamp (0-120 minutes) periods. (B) Rates of exogenous glucose infusion (Ginf) during the glucose clamp. All values are the mean ± SE.

period (Fig 2). After ICV leptin and during the glucose clamp, endogenous glucose production, calculated as the difference between the tracer-determined glucose Ra and glucose infusion rate, was similarly reduced from the baseline in both groups (4.4 ± 0.4 *v* 5.4 ± 0.3 mg · kg^{-1} · min^{-1}, AL *v* AV, P = NS; Fig 2). The exogenous glucose infusion rate required to clamp the plasma glucose level was also comparable in the two groups (12.9 ± 0.7 *v* 11.8 ± 0.6 mg · kg^{-1} · min^{-1}, AL *v* AV, P = NS; Fig 1). Similarly, glucose utilization rates were comparable between AL and AV groups in both the basal period (9.3 ± 0.3 and 9.5 ± 0.4 mg · kg^{-1} · min^{-1}, P = NS) and the glucose clamp period after ICV leptin and vehicle injection (17.0 ± 0.5 *v* 17.4 ± 0.4 mg · kg^{-1} · min^{-1}, P = NS; Fig 2).

Subacute ICV Leptin

Subacute ICV leptin ([SL] 10 μg bolus) moderately but insignificantly decreased overnight food consumption (14.1 ± 1.4 *v* 17.3 ± 1.9 g/rat, P = NS) and body weight (change, -2.5 ± 1.5 *v* 2.5 ± 1.9 g/rat, $P = .07$; Fig 3) compared with subacute vehicle (SV). Basal plasma glucose was reduced by SL (118.4 ± 1.9 mg/dL) as compared with SV (124.2 ± 1.8 mg/dL, $P < .05$). Plasma glucose levels were clamped at the respective baselines during the hyperinsulinemic-euglycemic clamp (118.6 ± 1.6 mg/dL for SL and 120.7 ± 1.0 mg/dL for SV, P = NS; Fig 4). The glucose infusion rate required to maintain the hyperinsulinemic-euglycemic clamp (120 to 180 minutes) was 43% greater with SL (16.9 ± 0.8 mg/kg/min) versus SV (11.8 ± 0.6, $P < .0001$; Fig 4). Basal glucose production rates were comparable for SL and SV (9.3 ± 0.4 *v* 9.6 ± 0.3 mg · kg^{-1} · min^{-1}, P = NS; Fig 5). The endogenous glucose production rate during the glucose clamp,

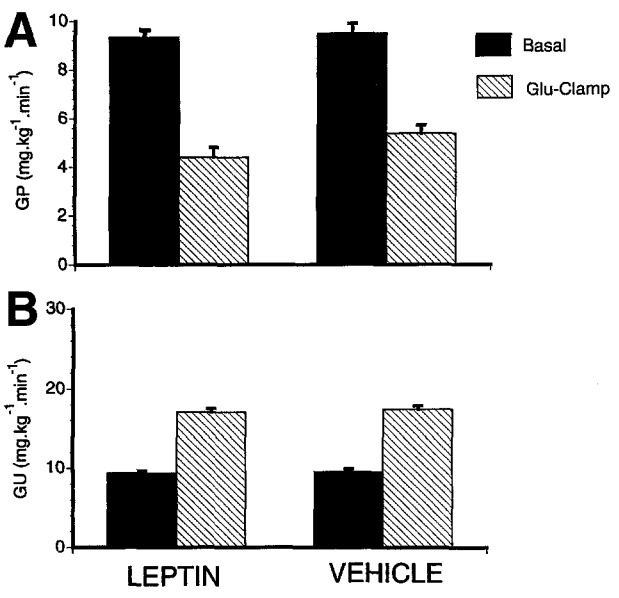

Fig 2. Tracer-determined (A) glucose production (GP) and (B) glucose utilization (GU) in acute ICV leptin and vehicle-treated rats during basal and glucose clamp (Glu-clamp) periods. During the glucose clamp period, GP was suppressed and GU was increased similarly in both the leptin and vehicle groups. There was no statistical difference between the 2 groups during both the basal and glucose clamp periods.

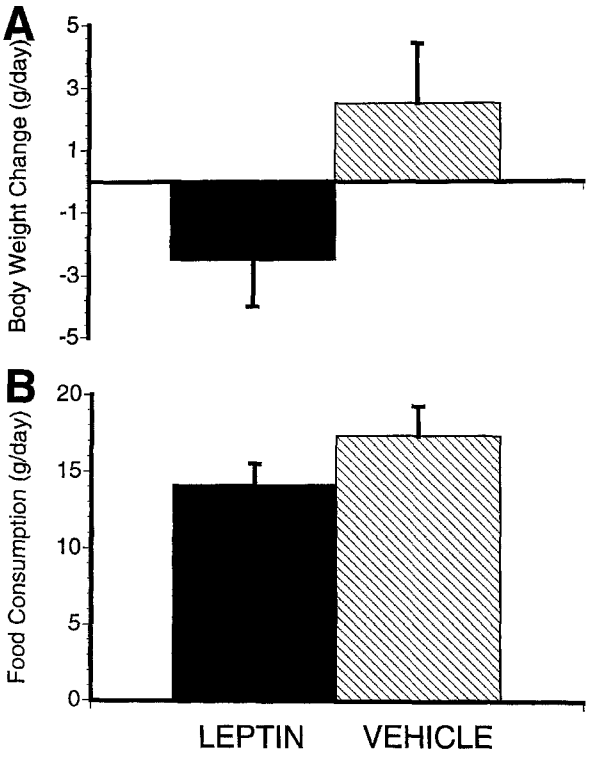

Fig 3. (A) Body weight and (B) food consumption changes in rats 1 day after bolus ICV leptin (10 μg, n = 6) or vehicle (n = 6) injection.

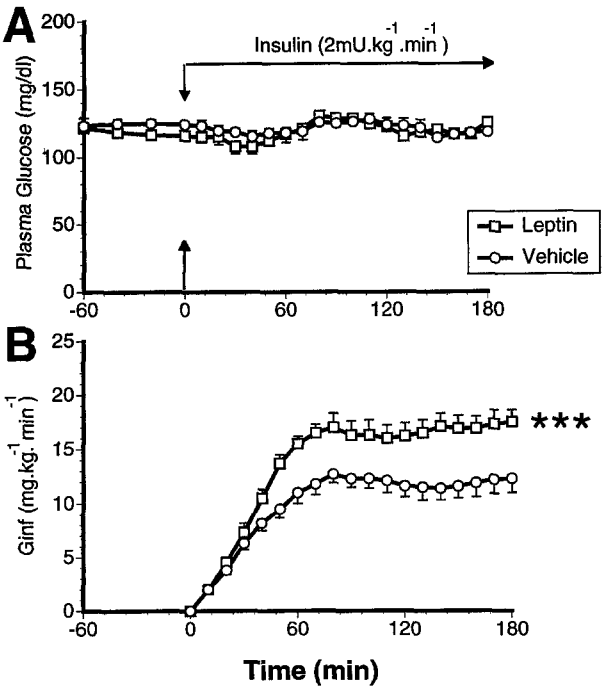

Fig 4. (A) Plasma glucose levels and (B) exogenous glucose infusion rates in rats after overnight bolus ICV leptin (10 μg, n = 6) or vehicle (n = 6) injection. Insulin infusion was initiated at 0 minutes and plasma glucose levels were clamped for 180 minutes. Glucose infusion rates are significantly different (*$P < .001$ *v* vehicle) during the last 60 minutes of the clamp.**

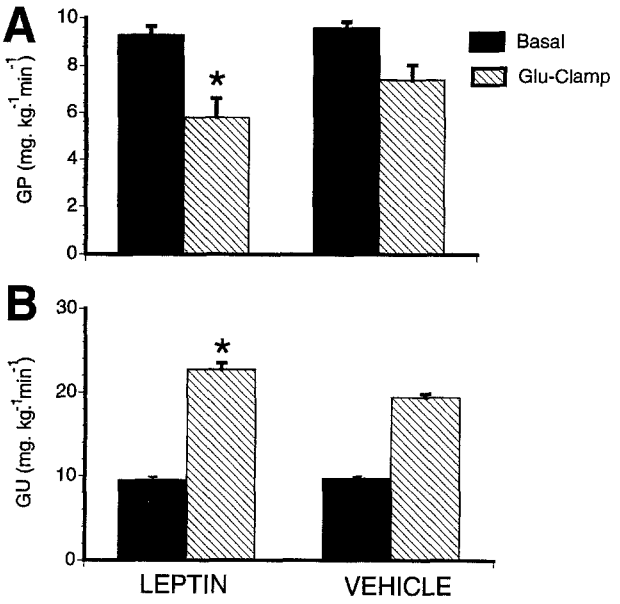

Fig 5. (A) Glucose production (GP) and (B) utilization (GU) rates during basal and glucose clamp periods in rats 1 day after bolus ICV leptin (10 μg, n = 6) or vehicle (n = 6) injection. *$P < .05$ *v* vehicle.

calculated as the difference between the tracer-determined glucose Ra and glucose infusion rate, while suppressed from the baseline ($P < .05$) in both groups, was suppressed to a significantly lower rate with SL versus SV (5.8 ± 0.5 *v* 7.4 ± 0.4 mg · kg^{-1} · min^{-1}, SL *v* SV, $P < .05$; Fig 5). Tracer-determined glucose utilization rates were comparable between SL and SV groups during the basal period (9.5 ± 0.4 and 9.7 ± 0.3 mg · kg^{-1} · min^{-1}, P = NS). During the glucose clamp period, glucose utilization was significantly greater with SL versus SV (22.7 ± 0.8 *v* 19.4 ± 0.7 mg · kg^{-1} · min^{-1}, $P < .005$; Fig 5). Plasma cholesterol, triglyceride, free fatty acid, β-hydroxybutyrate, lactate, glycerol, corticosterone, and insulin levels were not affected by an overnight bolus ICV injection of leptin (Table 1).

Chronic ICV Leptin

Chronic ICV leptin ([CL] 10 μg/d for 7 days) significantly reduced food intake (13.0 ± 1.1 g/d) compared with the chronic vehicle control ([CV] 18.7 ± 1.6 g/d, $P < .01$; Fig 6). Pair-fed (PF) animals were fed a daily ration equal to that consumed by the CL group. CL rats consistently lost body weight (change, −3.6 ± 0.5 g/d), which was highly significant compared with the small body weight gain in CV rats (change, 1.5 ± 1.0 g/rat, $P < .001$; Fig 6). PF animals also lost a significant amount of body weight (change, −2.6 ± 0.5 g/rat, $P < .05$ *v* CV), which was slightly less than but not significantly different from the loss in CL rats (P = NS). Baseline plasma glucose levels with CL were 109.9 ± 2.3 mg/dL, significantly lower ($P < .005$)

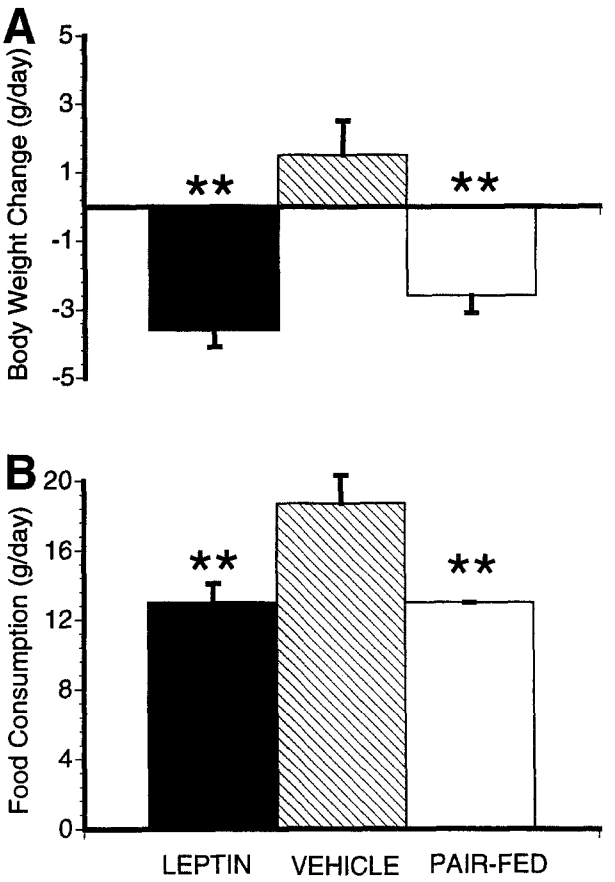

Fig 6. (A) Body weight and (B) food consumption changes in rats treated with chronic ICV leptin (10 μg/d, n = 6), vehicle (n = 5), or pair-feeding (n = 7) for 7 days. **$P < .01$ *v* vehicle.

than with CV (118.8 ± 3.3) and PF (118.4 ± 2.0 mg/dL; Fig 7). Plasma glucose levels were closely maintained at the respective baseline values during the glucose clamp (110.7 ± 2.3 for CL, 107.3 ± 1.0 for CV, and 115.7 ± 2.4 mg/dL for PF). The glucose infusion rate required to maintain the hyperinsulinemic-euglycemic clamp was 19.5 ± 1.6 mg · kg^{-1} · min^{-1} during the last hour (120 to 180 minutes) with CL, which was 64% greater than that of CV (11.9 ± 0.3, $P < .0001$) and nearly twofold that of PF (10.7 ± 0.8 mg · kg^{-1} · min^{-1}, $P < .0001$; Fig 7). The tracer-determined basal glucose production rate was moderately but insignificantly (P = .06) lower with CL (7.8 ± 0.4 mg · kg^{-1} · min^{-1}) versus CV (8.8 ± 0.3 mg · kg^{-1} · min^{-1}) and did not differ significantly versus PF (7.5 ± 0.3 mg · kg^{-1} · min^{-1}; Fig 8). CL treatment was associated with a significantly suppressed endogenous glucose production rate during the glucose clamp (3.5 ± 0.6 mg · kg^{-1} · min^{-1}), which was substantially lower than for CV (5.0 ± 0.3

Table 1. Plasma Metabolites and Hormones in Rats 1 Day After Bolus ICV Leptin or Vehicle (mean ± SEM)

Treatment	Cholesterol (mg/dL)	Triglyceride (mg/dL)	FFA (mEq/L)	β-OH-Butyrate (mg/dL)	Lactate (mg/dL)	Glycerol (mg/dL)	Corticosterone (μg/dL)	Insulin (pmol)
Leptin	67.2 ± 3.1	13.2 ± 1.7	0.5 ± 0.0	2.2 ± 0.2	2.4 ± 0.2	10.0 ± 0.0	8.6 ± 1.3	250 ± 66
Vehicle	65.2 ± 2.2	16.2 ± 2.8	0.6 ± 0.0	1.9 ± 0.2	2.0 ± 0.5	15.0 ± 0.3	12.8 ± 2.7	200 ± 33

NOTE. No statistical difference was found between the 2 groups for all of these parameters.

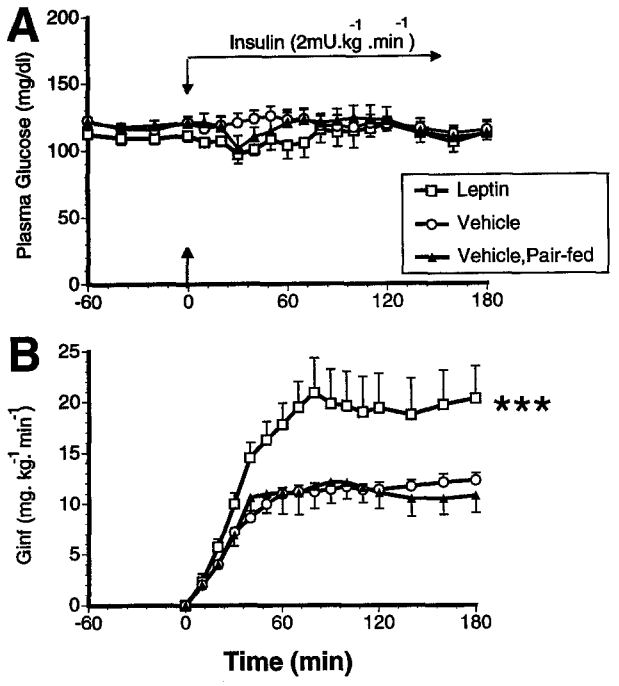

Fig 7. (A) Plasma glucose levels and (B) exogenous glucose infusion rates (Ginf) during basal (−60-0 minutes) and glucose clamp (0-180 minutes) periods in rats treated with ICV leptin (10 μg/d, n = 6), vehicle (n = 5), or vehicle and pair-feeding (n = 7) for 7 days. ***$P < .001$, leptin *v* vehicle and pair-fed groups during the last hour of the glucose clamp period (120-180 minutes).

mg · kg^{-1} · min^{-1}, $P < .005$) and PF (5.1 ± 0.5 mg · kg^{-1} · min^{-1}, $P < .001$; Fig 8). During the basal period, the whole-body glucose utilization rate was not significantly different with CL (7.9 ± 0.4 mg · kg^{-1} · min^{-1}) versus CV and PF (8.8 ± 0.3 and 7.6 ± 0.3 mg · kg^{-1} · min^{-1}, respectively). During the hyperinsulinemic-euglycemic clamp, CL treatment was associated with a threefold increase in whole-body glucose utilization (23.3 ± 1.8 mg · kg^{-1} · min^{-1}, $P < .001$ *v* CV and PF; Fig 8), whereas in both CV and PF, glucose utilization increased twofold (CV, 16.9 ± 0.2 mg · kg^{-1} · min^{-1}; PF, 17.1 ± 0.6 mg · kg^{-1} · min^{-1}) from the respective baseline values. CL treatment was associated with a marked decrease in the plasma triglyceride concentration and a significant increase in the plasma β-hydroxybutyrate concentration (Table 2). Circulating levels of cholesterol, triglyceride, free fatty acids, lactate, glycerol, corticosterone, and insulin were not significantly affected by CL. PF, similar to CL, resulted in a significant increase in β-hydroxybutyrate (Table 2).

DISCUSSION

This study demonstrates that r-metMuLeptin administered ICV enhances insulin sensitivity and whole-body glucose utilization in unrestrained conscious rats under postabsorptive conditions. These effects of leptin are not observed within the 2-hour experimental period in rats treated with AL, but are clearly demonstrable within 1 day after bolus ICV injection of leptin. The effects of leptin are most pronounced in rats treated with 7 days of continuous ICV infusion. With subacute and/or prolonged ICV administration, leptin also significantly enhanced the effect of insulin in suppressing hepatic glucose production.

It has also been reported that leptin infused intravenously for 6 hours[6,8] and subcutaneously for 8 days[7] substantially augments the effect of insulin in the suppression of hepatic glucose production. Interestingly, a minute dose of leptin given centrally in the current study also resulted in augmented suppression of hepatic glucose production during a hyperinsulinemic-euglycemic clamp, suggesting involvement of the central nervous system in the regulation of glucose production. The inability of AL to affect glucose production within 2 hours is presumably due to the insufficient time for leptin action, since a bolus of leptin overnight was able to sufficiently augment insulin's inhibition of glucose production. Leptin's effect on hepatic glucose production was found to be associated with a reduction in hepatic glycogenolysis and changes in glucokinase, phosphoenolpyruvate carboxykinase, and glucose-6-phosphatase activity.[6-8]

The decrease of triglyceride concentrations in the circulation after subacute and chronic ICV leptin treatment are in agreement with recent reports that leptin causes a marked depletion in body fat[25,26] and an exaggerated rate of free fatty acid oxidation.[26] Hyperleptinemia induced in normal rats by adenovirus gene transfer did not cause an increase in the plasma β-hydroxybutyrate concentration.[26] In our study, plasma β-hydroxybutyrate increased in rats treated with 7-day ICV leptin infusion, reflecting augmented fat utilization and ketogenesis, but not with acute or overnight injections. The fasting state in our experimental animals, necessary for the glucose turnover studies, may have contributed to the increase in ketone bodies in leptin-treated and PF rats in addition to their reduced food intake.

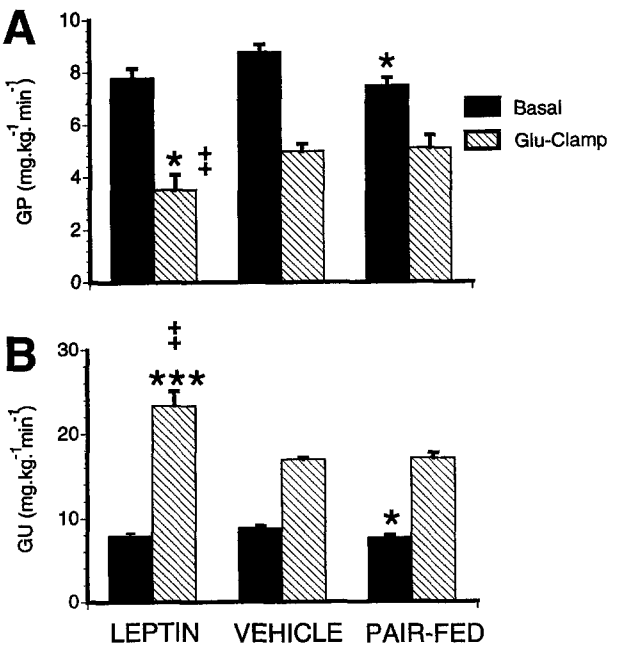

Fig 8. (A) Glucose production (GP) and (B) utilization (GU) rates during basal and glucose clamp periods in rats treated with chronic ICV leptin (10 μg/d, n = 6), vehicle (n = 5), or vehicle and pair-feeding (n = 7) for 7 days. $P < .05$, *$P < .01$, and ***$P < .001$, leptin or pair-fed group *v* vehicle; ‡$P < .01$ *v* pair-fed.

Table 2. Plasma Metabolites and Hormones in Rats Treated With 7-Day ICV Leptin, Vehicle, and Pairfeeding (mean ± SEM)

Treatment	Cholesterol (mg/dL)	Triglyceride (mg/dL)	FFA (mEq/L)	β-OH-Butyrate (mg/dL)	Lactate (mg/dL)	Glycerol (mg/dL)	Corticosterone (μg/dL)	Insulin (pmol)	Leptin (ng/mL)
Leptin	64.8 ± 6.2	17.67 ± 1.2*	0.6 ± 0.0	3.07 ± 0.7*	3.7 ± 0.3	16.3 ± 1.2	25.9 ± 4.1	250 ± 60	1.4 ± 0.5
Vehicle	72.0 ± 3.6	44.4 ± 7.6	0.7 ± 0.1	1.2 ± 0.3	3.5 ± 0.3	19.4 ± 2.7	26.9 ± 5.4	483 ± 133	0.6 ± 0.2
Pair-fed	60.6 ± 3.5	39.14 ± 8.7	0.4 ± 0.2	2.67 ± 0.4*	2.7 ± 0.3	16.1 ± 2.2	19.0 ± 1.1	300 ± 66	0.5 ± 0.2

*$P < .05$ *v* vehicle.

Several recent studies have reported the effects of leptin on systemic glucose utilization in rodents. One study[27] reported that ICV leptin, similar to intravenous leptin, stimulated systemic glucose turnover and muscle glucose uptake in normal mice under basal conditions without a glucose clamp. Leptin's effect on insulin sensitivity was not examined in that study. Another study reports an increased glucose infusion rate during a glucose clamp in the anesthetized rat treated with subcutaneous infusion of leptin for 48 hours.[28] Anesthesia and surgery can result in a general state of stress, which is known to decrease glucose utilization,[29] and hepatic glucose production is substantially stimulated under various stress conditions.[30] It is recently reported that leptin per se may be a stress-related hormone that is decreased in critically ill patients, suggesting a metabolic adaptation.[31] The present study is the first to demonstrate that in the conscious unrestrained normal rat, centrally administered leptin markedly enhances systemic insulin sensitivity and glucose utilization in the postabsorptive state. We also demonstrate that the effects of leptin are achieved by doses that are hundreds-fold lower than those used for peripheral administration, and that a clear-cut enhancement in insulin sensitivity and glucose utilization can be obtained overnight following a bolus injection. It can be deduced that some changes may be taking place even earlier within this time frame.

The mechanism for the enhancement in insulin sensitivity and glucose utilization has yet to be identified. There is in vitro and ex vivo evidence suggesting that leptin can act directly on specific peripheral tissues such as hepatocytes,[32] islet cells,[26] adipocytes,[33] and skeletal muscle cells.[25] However, the involvement of the central nervous system as a key link in the in vivo actions of leptin has been agreed upon by many investigators.[4,10,27,34] It is very likely that many of the effects observed after peripheral administration of leptin are due to the interaction of leptin with the receptors in the brain. In humans, leptin receptors have been found concentrated in the neurons of the hypothalamus, in addition to the choroid plexus and a few other loci in the brain.[35] Stimulation of the ventromedial hypothalamus neurons with chemical and electrical stimuli has produced profound increases in glucose utilization in the brown adipose tissue, heart, and skeletal muscle.[36] It has been shown that cold exposure stimulates glucose uptake in brown adipose tissue, as well as skeletal muscle, suggesting the use of glucose as an energy substrate in shivering (muscle) and nonshivering (brown adipose tissue) thermogenesis.[37] Such regulation is likely achieved by activation of the hypothalamus, which is known to regulate thermogenic homeostasis. In the present study, leptin was administered centrally, which yielded no detectable increase of the hormone in the peripheral circulation after even 7 days of treatment. The central-action mechanism of leptin effects is further supported by the leptin doses used in the current studies, which are several hundred-fold smaller than those used effectively in peripheral administration studies.[6-8] Such small doses do not cause any discernible difference in circulating leptin levels compared with the vehicle control in this study. Furthermore, there is very little of the biologically active form (form B) of the leptin receptor in tissues other than the brain regions. Leptin's effects on food intake and body weight reduction could influence systemic glucose utilization, but in the present study are not likely the primary factors. This is because food restriction and body weight reduction in PF, vehicle-treated animals did not lead to any enhancement in glucose utilization in response to insulin as compared with the vehicle-treated control animals. In fact, food deprivation has been shown to induce insulin resistance rather than insulin sensitivity in the rat.[37,38]

In summary, we have demonstrated that ICV administration of r-metMuLeptin results in significant increases in insulin sensitivity and systemic glucose utilization. These effects of leptin are achieved as early as 16 to 20 hours after leptin administration. Centrally administered leptin also significantly enhanced the insulin-induced suppression of hepatic glucose production, which is indicative of increased hepatic sensitivity to insulin and may contribute to the decrease in fasting plasma glucose in rats with CL treatment. Owing to the small dose used in the study and the unchanged plasma leptin levels, the effects of leptin are not likely mediated by peripheral leptin actions. The effects of leptin appear to be associated with its interactions with leptin receptors in the central nervous system.

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REFERENCES

1. Zhang Y, Proenca R, Maffei M, et al: Positional cloning of the mouse obese gene and its human homologue. Nature 372:425-431, 1994

2. Pelleymounter M, Cullen M, Baker M, et al: Effects of the obese gene product on body weight regulation in ob/ob mice. Science 269:540-543, 1995

3. Halaas JL, Gajiwala KS, Maffei M, et al: Weight-reducing effects of the plasma protein encoded by the obese gene. Science 269:542-546, 1995

4. Campfield LA, Smith FJ, Guisez Y, et al: Recombinant mouse OB protein: Evidence for a peripheral signal linking adiposity and central neural networks. Science 269:546-549, 1995

5. Levin N, Nelson C, Gurney A, et al: Decreased food intake does not completely account for adiposity reduction after ob protein infusion. Proc Natl Acad Sci USA 93:1726-1739, 1996

6. Barzilai N, Massilon D, Vuguin P, et al: Leptin acutely modulates hepatic gene-expression, glucose fluxes, and insulin action. Diabetes 46:256, 1997 (suppl 1, abstr)

7. Barzilai N, Wang J, Massilon D, et al: Leptin selectively decreases visceral adiposity and enhances insulin action. J Clin Invest 100: 3105-3110, 1997

8. Rossetti L, Massilon D, Barzilai N, et al: Short term effects of leptin on hepatic gluconeogenesis and in vivo insulin action. J Biol Chem 272:27758-27763, 1997

9. Berti L, Kellerer M, Capp E, et al: Leptin stimulates glucose transport and glycogen synthesis in C_2C_{12} myotubules: Evidence for a PI3-kinase mediated effect. Diabetologia 40:606-609, 1997

10. Halaas JL, Boozer C, Blair-West J, et al: Physiological response to long-term peripheral and central leptin infusion in lean and obese mice. Proc Natl Acad Sci USA 94:8878-8883, 1997

11. Niskanen L, Haffner S, Karhumen LJ, et al: Serum leptin in relation to resting energy expenditure and fuel metabolism in obese subjects. Int J Obes 21:309-313, 1997

12. Lonnqvist F, Arner P, Nordfors L, et al: Overexpression of the obese (ob) gene in adipose tissue of human obese subjects. Nat Med 1:950-953, 1995

13. Hamilton BS, Paglia D, Kwan AYM, et al: Increased obese mRNA expression in omental fat cells from massively obese humans. Nat Med 1:953-956, 1995

14. Scheen AJ, Paquot N, Letiexhe MR, et al: Glucose metabolism in obese subjects: Lessons from OGTT, IVGTT and clamp studies. Int J Obes 19:14-20, 1995 (suppl 3)

15. DeFronzo RA, Bonadonna RC, Ferrannini E: Pathogenesis of NIDDM: A balanced overview. Diabetes Care 15:318-368, 1992

16. Paxinos G, Watson C: The Rat Brain in Stereotaxic Coordinates (ed 2). San Diego, CA, Academic, 1986

17. Shi ZQ, Rastogi KS, Lekas M, et al: Glucagon response to hypoglycemia is improved by insulin-independent restoration of normoglycemia in diabetic rats. Endocrinology 137:3193-3199, 1996

18. Fisher DA, Brown MR: Somatostatin analog:plasma catecholamine suppression mediated by the central nervous system. Endocrinology 107:714-718, 1980

19. Miles PD, Yamatani K, Lickley HLA, et al: Mechanism of glucoregulatory responses to stress and their deficiency in diabetes. Proc Natl Acad Sci USA 88:1296-1300, 1991

20. Ferrannini E, Natali A, Bell P, et al: Insulin resistance and hypersecretion in obesity. J Clin Endocrinol Metab 100:1166-1173, 1997

21. Steele R, Wall JS, deBodo RC, et al: Measurement of size and turnover rate of body glucose pool by isotope dilution method. Am J Physiol 187:15-24, 1956

22. Radziuk J, Norwich KH, Vranic M: Experimental validation of measurements of glucose turnover in nonsteady state. Am J Physiol 234:E84-E93, 1978 (suppl)

23. Finegood DT, Bergman RN: Optimal segments. A method for smoothing tracer data to calculate metabolic fluxes. Am J Physiol 244:E472-E479, 1983 (suppl)

24. Wu-Peng XS, Chua SC, Okada N, et al: Phenotype of the obese Koletsky (f) rat due to Tyr763 stop mutation in the extracellular domain of the leptin receptor (Lepr.). Diabetes 46:513-518, 1997

25. Muoio DM, Dohm GL, Fiedorek FT, et al: Leptin directly alters lipid partitioning in skeletal muscle. Diabetes 46:1360-1363, 1997

26. Shimabukuro M, Koyama K, Chen G, et al: Direct antidiabetic effect of leptin through triglyceride depletion of tissues. Proc Natl Acad Sci USA 94:4637-4641, 1997

27. Charron ML, Friedman J: Acute intravenous and intracerebroventricular leptin infusion increases glucose uptake in insulin sensitive tissues and glucose turnover by an insulin independent pathway. J Physiol 389:374-377, 1997

28. Sivitz WI, Walsh SA, Morgan DA, et al: Effects of leptin on insulin sensitivity in normal rats. Neuroendocrinology 138:3395-3401, 1997

29. Tsubo T, Kudo T, Matsuki A, et al: Decreased glucose utilization during prolonged anesthesis and surgery. Can J Anaesth 37:645-649, 1990

30. Taborsky GJ Jr, Porte D Jr, Brown MR, Koob GF, Rivier C (eds: Stress Neurobiology and Neuroendocrinology. New York, NY, Dekker, 1991, pp 519-548

31. Bornstein SR: Is leptin a stress related peptide? Nat Med 3:937, 1997

32. Cohen B, Novick D, Rubinstein M: Modulation of insulin activities by leptin. Science 274:1185-1188, 1996

33. Muller G, Ertl J, Gerl M, et al: Leptin impairs metabolic actions of insulin in isolated rat adipocytes. Science 272:10585-10593, 1997

34. Kamohara S, Burcelin R, Halaas JL, et al: Acute stimulation of glucose metabolism in mice by leptin treatment. Nature 389:374-377, 1997

35. Couce ME, Burguera E, Parisi JE, et al: Localization of leptin receptor in the human brain. Neuroendocrinology 66:145-150, 1997

36. Sudo M, Minokoshi Y, Shimazu T: Ventromedial hypothalamic stimulation enhances peripheral glucose uptake in anesthetized rats. Am J Physiol 261:E298-E303, 1991 (suppl)

37. Shibata H, Perusse F, Vallerand A, et al: Cold exposure reverses inhibitory effects of fasting on peripheral glucose uptake in rats. Am J Physiol 257:R96-R101, 1989 (suppl)

38. Penicaud L, Kande JK, Le Magnen J, et al: Insulin action during fasting and refeeding in rat determined by euglycemic clamp. Am J Physiol 249:E514-E518, 1985 (suppl)