Inhibitory Effects of Leptin-Related Synthetic Peptide 116–130 on Food Intake and Body Weight Gain in Female C57BL/6J *ob/ob* Mice May Not Be Mediated by Peptide Activation of the Long Isoform of the Leptin Receptor

Patricia Grasso, David W. White, Louis A. Tartaglia, Matthew C. Leinung, and Daniel W. Lee

We recently reported that intraperitoneal administration of leptin-related synthetic peptide 116–130 [LEP-(116–130)] resulted in reduced food intake and significant weight loss in homozygous female C57BL/6J *ob/ob* mice. In this study, we used two in vitro bioassays to show that the interaction of LEP-(116–130) with the long form of the leptin receptor (OB-R_b), the receptor isoform that is predominantly expressed in the hypothalamus, is not required for the observed in vivo effects of the peptide on energy balance. LEP-(116–130) was unable to compete the binding of alkaline phosphatase-leptin fusion protein to OB-R. Moreover, LEP-(116–130) was unable to activate signal transduction by OB-R_b in vitro. In homozygous female C57BLKS/J-m *db/db* mice that do not express OB-R_b, intraperitoneal administration of LEP-(116–130) reduced body weight gain and blood glucose levels but not food intake, which further supports a mechanism of action that does not require peptide interaction with OB-R_b. *Diabetes* 48:2204–2209, 1999

Leptin, the product of the *ob* gene, is a 16-kDa secreted protein that is produced by white adipocytes and placenta and functions as an afferent signal to influence energy homeostasis through effects on energy intake and expenditure (1–4). Central or peripheral administration of exogenous leptin to leptin-deficient obese *ob/ob* mice results in a dose-dependent loss of adipose tissue mass (2,4–6). The weight-reducing effects of leptin are likely mediated through interaction with a receptor for leptin (OB-R), the product of the *db* gene (7), which is expressed predominantly in the hypothalamus, a region of the brain associated with regulation of body weight (8,9).

Structural and functional data indicate that OB-R is a member of the class I cytokine receptor superfamily (7). Members of this class of receptors lack intrinsic tyrosine kinase activity and are activated by ligand-induced receptor homo- or heterodimerization and activation of receptor-associated members of the Janus kinase (JAK) family (10,11). Activated JAK tyrosine kinases rapidly phosphorylate and activate cytoplasmic target proteins, including the signal transducers and activators of transcription (STAT) family of transcription factors (11). Leptin binding by OB-R mediates ligand-induced phosphorylation and the activation of the DNA binding activity of STAT1, STAT3, and STAT5 (12).

At least five isoforms of the OB-R exist that are generated by alternative mRNA splicing (13). In most tissues, the OB-R mRNAs encode three transmembrane proteins, each with an extracellular domain of 814 amino acids and a short cytoplasmic domain of 32–40 amino acid residues (OB-R_a, OB-R_c, and OB-R_d) (4). The predominant isoform is OB-R_a (or OB-R_s). In the hypothalamus, OB-R mRNA is expressed as a protein with an extracellular domain that is identical to that of OB-R_a, OB-R_c, and OB-R_d but has a 302–amino acid cytoplasmic domain (OB-R_b or OB-R_l) through which leptin signaling occurs (12,13). A soluble leptin receptor (OB-R_e) that lacks a transmembrane region has also been identified (12). Mutation of the *db* gene results in the production of an aberrant splice product of the OB-R_b transcript and encodes a receptor with a truncated cytoplasmic domain that is incapable of leptin signaling (13,14).

The results of this study provide both in vitro and in vivo evidence that the effects of leptin-related synthetic peptide 116–130 [LEP-(116–130)], which we have previously shown to reduce food intake and body weight gain in female C57BL/6J *ob/ob* mice (15), do not require peptide binding by OB-R or activation of OB-R_b. Although the mechanism of action by which LEP-(116–130) exerts its effects on energy balance is unknown, clearly this mechanism is different from that by which the leptin signal is transduced. This observation suggests that LEP-(116–130) or analogs thereof may have potential use in the development of alternative therapeutic approaches for the treatment of human obesity and its associated dysfunctions.

From the Department of Biochemistry and Molecular Biology (P.G., D.W.L.) and the Department of Medicine (P.G., M.C.L., D.W.L.), Albany Medical College, Albany, New York; and Millennium Pharmaceuticals (D.W.W., L.A.T.), Cambridge, Massachusetts.

Address correspondence and reprint requests to Dr. Patricia Grasso, Department of Biochemistry and Molecular Biology, Albany Medical College, MC-10, Albany, NY 12208. E-mail: pgrasso@ccgateway.amc.edu.

Received for publication 16 March 1999 and accepted in revised form 13 July 1999.

D.W.W. and L.A.T. hold stock in Millennium Pharmaceuticals.

AP-OB, alkaline phosphatase-human leptin; HRRE, hematopoietin receptor response; JAK, Janus kinase; LEP-(116–130), leptin-related synthetic peptide 116–130; LEP-(146–160), leptin-related synthetic peptide 146–160; OB-R, leptin receptor; PBS, phosphate-buffered saline; QCB, Quality Controlled Biochemicals; SEAP, secreted alkaline phosphatase; STAT, signal transducers and activators of transcription.

RESEARCH DESIGN AND METHODS

Peptide synthesis, purification, and characterization. LEP-(116–130) and leptin-related synthetic peptide 146–160 [LEP-(146–160)] were synthesized with the solid phase method (16) as peptide amides by Quality Controlled Biochemicals (QCB, Hopkinton, MA). Fluorenylmethoxycarbonyl-protected L-amino acids were used. The peptide amides were purified to >95%, evaluated by reversed-phase high-performance liquid chromatography, and were represented as single peaks in the chromatographic profiles (QCB). Fidelity of synthesis was confirmed by mass spectral analysis (QCB).

Cell culture. COS-7 and GT1–7 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum as previously described (17,18).

Expression vectors and secreted alkaline phosphatase reporter gene constructs. The expression vectors for OB-R_a and OB-R_b have been previously described (19,20). The reporter gene construct pHRRE-SEAP (hematopoietin receptor response element-secreted alkaline phosphatase) was generated by subcloning the hemopoietin receptor response element into the pSEAP-Promoter (CLONTECH, Palo Alto, CA) as reported previously (19). The luciferase reporter gene construct pGL3 was commercially obtained from Promega (Madison, WI).

Cell transfection and analysis. COS-7 and GT1–7 cells were transfected by the lipofectamine method (12). For SEAP assays, GT1–7 cells were transfected with 1 µg pHRRE-SEAP, 0.5 µg pGL3, and 3 µg OB-R_b receptor constructs. At 48 h after transfection, cultures were washed twice with serum-free medium and stimulated with 6 nmol/l mouse leptin or increasing concentrations of LEP-(116–130) or LEP-(146–160) for 24 h in nonsupplemented cell culture medium. LEP-(146–160), which has been shown to have no activity in vivo (15), was used as a negative control. Supernatants were collected, and SEAP reporter activities were measured via chemiluminescence with the GreatEscApe alkaline phosphatase detection kit as described by the manufacturer (CLONTECH). Luminescence was measured in a Microbeta plus scintillation counter (Wallac, Gaithersburg, MD) and was expressed as arbitrary units of luminescence activity. Cell lysates were generated with a luciferase assay system kit (Promega), and luciferase activities were measured with a microplate luminator (Tropix, San Francisco, CA).

Analysis of LEP-(116–130) binding. COS-7 cells were transfected with expression vector for OB-R_a. Two days after transfection, the cells were incubated with 1 nmol/l alkaline phosphatase-human leptin (AP-OB) fusion protein in the absence or presence of 30 nmol/l mouse leptin, 300 µmol/l LEP-(116–130), or 300 µmol/l LEP-(146–160). Bound AP activity was determined as previously described (21).

Animal procedures

Housing. Female C57BLKS/J-m wild-type (+/+) and homozygous diabetic mice (*db/db*) 6 weeks of age (Jackson Laboratory, Bar Harbor, ME) were maintained in a temperature-controlled room (24°C) in the Albany Medical College Animal Resources Facility under alternating 12-h light and dark periods (light period 0700–1900). The animals were housed three per side in 30 cm × 30 cm × 15 cm split polycarbonate cages fitted with stainless steel wire lids and air filters and were supported on ventilated racks.

Peptide administration. LEP-(116–130) and LEP-(146–160) were dissolved in sterile phosphate-buffered saline (PBS) (pH 7.2) administered daily for 7 days between 1500 and 1600 in a single 1 mg/0.2 ml i.p. injection. Control mice received 0.2 ml i.p. PBS only.

Feeding and weighing schedule. On day 1 of the study and on each day thereafter, 200 g of pellet rodent diet were added to the hopper on each side of the cages between 0900 and 1100. Food remaining after 24 h was weighed to the nearest 0.1 g, and the average amount of food consumed per mouse was calculated in means ± SE (n = 6). The mice drank water ad libitum throughout the study. The mice were weighed once daily between 0900 and 1100 on an Acculab V-133 electronic balance (Cole-Parmer, Vernon Hills, IL).

Measurement of blood glucose and thermoregulatory studies. Blood was drawn from the tail vein of each mouse 2 h before the onset of the dark period at the beginning of the study (day 0) and after 2, 4, and 6 days of treatment with 1 mg/day i.p. LEP-(116–130). Blood glucose levels were determined with a Glucometer Elite (Bayer, Elkhart, IN) blood glucose monitor.

After 4 and 7 days of treatment with 1 mg/day i.p. LEP-(116–130), sensitivity to cold was examined by placing the mice without food or water in a cold room with an ambient temperature of 4°C. Body temperature was measured with a rectal probe every hour for 4 h.

Euthanasia. Euthanasia was performed via pentobarbitol injection (100 mg/kg body weight i.p.). These animal procedures were reviewed and approved by the Animal Care and Use Committee of the Albany Medical College and are in accordance with institutional guidelines.

Statistical analysis. Changes in body weight, differences in food intake, blood glucose levels, and body temperature for peptide-treated and vehicle-injected control mice were analyzed with a two-tailed Wilcoxon signed-rank test and were considered significant at $P < 0.05$.

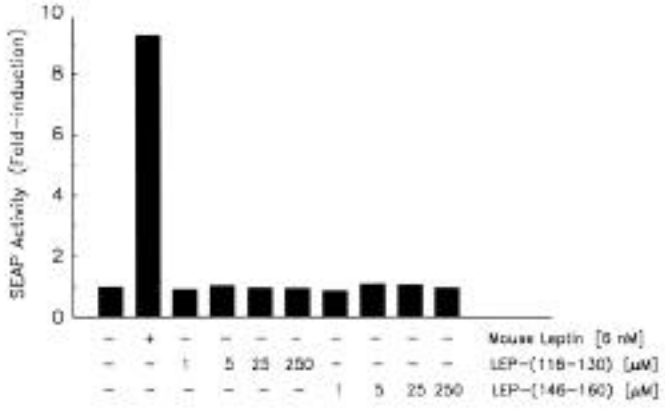

FIG. 1. Effects of LEP-(116–130) on signal transduction by OB-R_b. GT1–7 cells were cotransfected with the reporter gene construct HRRE-SEAP and cDNA encoding murine OB-R_b. Two days after transfection, cultures were mock stimulated (in growth medium alone) or treated with 6 nmol/l mouse leptin or increasing concentrations of LEP-(116–130) or LEP-(146–160) for 24 h and then analyzed for reporter gene induction. The culture medium was harvested, and SEAP activity was determined. SEAP values have been normalized to levels of firefly luciferase activity expressed from a cotransfected marker plasmid (pGL3).

RESULTS

Effects of LEP-(116–130) on signal transduction and ligand binding by OB-R. OB-R exhibits homology with members of the class I cytokine receptor superfamily. Consistent with this prediction, recorder-based assays and gel-shift experiments have documented that OB-R_b can mediate activation of STAT1, STAT3, and STAT5 and can stimulate transcription through both interleukin-6 and hematopoietin receptor response elements (12). Because the HRRE recorder used for these assays is able to measure changes in the activation of multiple STATs (including STAT1, STAT3, and STAT5), we used this recorder-based system to determine whether LEP-(116–130) could activate OB-R_b signaling.

GT1–7 cells were cotransfected with the reporter gene construct HRRE-SEAP and cDNA encoding OB-R_b and were analyzed for reporter gene activation in the absence or presence of mouse leptin (6 nmol/l) or increasing concentrations (1–250 µmol/l) of LEP-(116–130) (Fig. 1). In the absence of ligand stimulation, transfected cells showed minimal reporter gene activity. SEAP activity was increased 11-fold by 6 nmol/l mouse leptin. LEP-(116–130) was unable to stimulate SEAP activity above basal levels at all concentrations tested. LEP-(146–160), which is inactive in vivo (15), was also incapable of inducing an increase in reporter gene activity.

The effects of LEP-(116–130) on ligand binding by OB-R are shown in Fig. 2. COS-7 cells transfected with OB-R_a exhibit a large increase in AP-OB binding activity relative to mock transfected cells. Bound AP-OB was efficiently competed with the addition of 30 nmol/l unlabeled mouse leptin in the binding assay. In contrast, neither LEP-(116–130) nor LEP-(146–160) at a dose of 300 µmol/l was able to inhibit AP-OB binding.

Effects of LEP-(116–130) on body weight and food intake in female C57BLKS/J-m *db/db* mice. The results of our in vitro studies suggest that the observed in vivo effects of LEP-(116–130) on body weight gain and food intake in female *ob/ob* mice (15,16) were not mediated by pep-

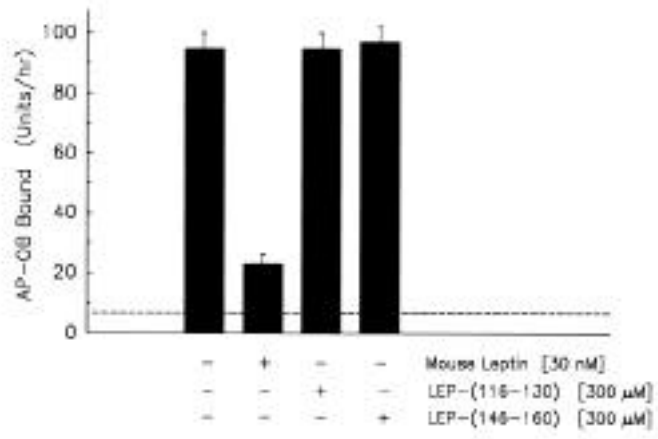

FIG. 2. Effects of LEP-(116–130) on ligand binding by OB-R_a. COS-7 cells were mock transfected or transfected with cDNA encoding OB-R_a. Forty-eight hours after transfection, the cells were incubated in 1 nmol/l human AP-OB fusion protein in the absence or presence of 30 nmol/l mouse leptin, 300 µmol/l LEP-(116–130), or 300 µmol/l LEP-(146–160). Bound AP-OB activity was determined. The bars represent the average of two binding measurements; the vertical lines represent the differences between the two binding measurements. ––, Basal levels of ligand binding by mock transfected cells.

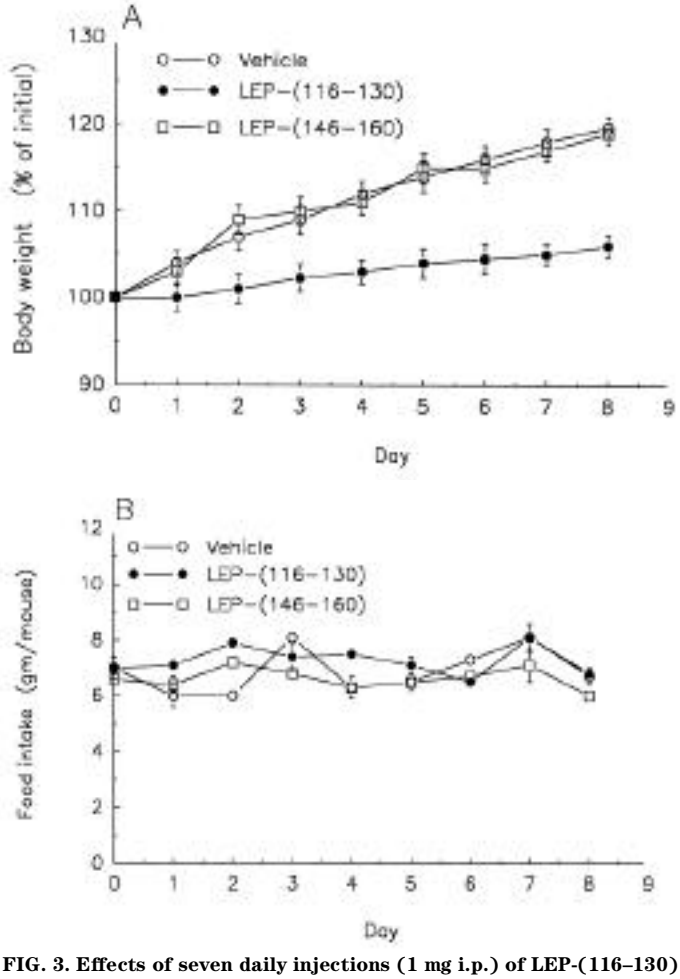

FIG. 3. Effects of seven daily injections (1 mg i.p.) of LEP-(116–130) or LEP-(146–160) on body weight gain and food intake of female C57BLKS/6-m *db/db* mice. The graph shows changes in body weight (expressed as a percentage of initial weight) (*A*) and food intake (expressed as grams of food consumed per mouse) (*B*) in mice treated with vehicle, LEP-(116–130), or LEP-(146–160). Data are means ± SE for changes in body weight or food intake in a group of six mice.

tide activation of hypothalamic OB-R_b. To obtain further support for this theory, we examined the effects of LEP-(116–130) on food intake and body weight gain in female *db/db* mice, which are genetically deficient in functional OB-R_b. Daily administration of 1 mg i.p. LEP-(116–130) for 7 consecutive days resulted in a reduced rate of body weight gain (Fig. 3*A* and Table 1) in the presence of increased food intake (Fig. 3*B* and Table 2) compared with vehicle-injected control mice. LEP-(146–160) (1 mg/day i.p. for 7 days) had no effect on either body weight (Fig. 3*A* and Table 1) or food consumption (Fig. 3*B* and Table 2).

Effects of LEP-(116–130) on blood glucose and thermogenesis in female C57BLKS/J-m *db/db* mice. We attempted to determine whether the effects of LEP-(116–130) on body weight gain and food intake in *db/db* mice were associated with changes in glucose utilization. The results of LEP-(116–130) on blood glucose levels after 2, 4, and 6 days of treatment are shown in Fig. 4. Although glucose levels in vehicle-injected control mice were consistently high throughout the study (556–600 mg/dl), blood glucose levels were significantly reduced ($P < 0.05$) by ~100 mg/dl after 2 days of LEP-(116–130) treatment and were sustained throughout the 7 days of the study (454–470 mg/dl). When the mice were withdrawn from treatment, blood glucose levels returned to the level of vehicle-injected control mice within 24 h (data not shown).

The apparent dissociation between food intake and body weight gain observed in mice treated with LEP-(116–130) suggests that the peptide may influence thermogenesis. To test this hypothesis, we subjected vehicle-injected control mice and mice receiving LEP-(116–130) for 4 or 7 days to cold stress (4°C) and assessed their responses to low ambient temperature. Abnormalities in thermoregulation were evident in both vehicle-injected control mice and mice treated with LEP-(116–130) for 4 (Fig. 5*A*) or 7 (Fig. 5*B*) days. Wild-type (+/+) mice maintained a core body temperature of approximately 35.2 ± 1.7°C during the 4 h exposure; *db/db* mice injected with vehicle or LEP-(116–130) for 4 and 7 days exhibited a marked decrease in core temperature after 1 h at 4°C that was further reduced by continued exposure for an additional 3 h.

Effects of LEP-(116–130) on body weight, food intake, blood glucose, and thermogenesis in female wild-type (+/+) C57BLKS/J-m mice. The effects of LEP-(116–130) on body weight and food intake in female wild-type (+/+)

TABLE 1
Effects of LEP-(116–130) and LEP-(146–160) (1 mg/day i.p.) on body weight gain in female *db/db* mice after 7 days of treatment

	Vehicle	LEP-(116–130)	LEP-(146–160)
Initial weight (g)	29.8 ± 1.5	30.8 ± 1.6	30.6 ± 1.6
Weight after 7 days (g)	35.7 ± 1.6	32.6 ± 1.8	36.2 ± 1.4
Weight increase (g)	5.9	1.8	5.6
Increase (%)	19.8	5.8	18.3
*P**		0.047	0.46

Data are means ± SE or % ($n = 6$). *The mean of the differences between the initial and 7-day body weight of each mouse ($n = 6$) in peptide-treated groups was compared with the mean of the differences between the initial and 7-day body weight of each vehicle-injected control mouse ($n = 6$). The two-tailed *P* values were determined with the Wilcoxon signed-rank test.

TABLE 2
Cumulative effects of LEP-(116–130) and LEP-(146–160) (1 mg/day i.p.) on food intake in female *db/db* mice after 7 days of treatment

Treatment	Cumulative food intake (g/mouse)
Vehicle	54.0 ± 0.3
LEP-(116–130)	64.9 ± 0.2*
LEP-(146–160)	53.5 ± 0.3

Data are means ± SE. *P = 0.021. The cumulative food intake by mice (n = 6) in the group treated with LEP-(116–130) was compared with the cumulative food intake by vehicle-injected control mice (n = 6). The two-tailed P value was determined with the Wilcoxon signed-rank test.

C57BLKS/J-m mice are summarized in Tables 3 and 4, respectively. In a pattern similar to that observed in *db/db* mice (Tables 1 and 2), daily administration of 1 mg i.p. LEP-(116–130) for 7 days resulted in a significantly ($P < 0.05$) reduced rate of body weight gain (Table 3) that occurred in the presence of increased (but not statistically significant) food intake (Table 4) compared with vehicle-injected control mice.

Blood glucose levels in vehicle-injected wild-type (+/+) C57BLKS/J-m mice ranged from 125 ± 2.7 to 146 ± 2.4 mg/dl throughout the 7 days of the study. These values were not significantly changed by treatment for 7 days with 1 mg i.p. LEP-(116–130) and ranged from 127 ± 1.6 to 149 ± 1.1 mg/dl.

Administration of LEP-(116–130) to wild-type (+/+) C57BLKS/J-m mice for 4 or 7 days had no effect on their ability to thermoregulate. When subjected to cold stress for 4 h at 4°C, the core temperatures of vehicle-injected control mice fell from 32.3 ± 0.4 to 26.4 ± 1.1°C. The core temperatures of mice given LEP-(116–130) fell from 31.8 ± 0.3 to 28.2 ± 0.4°C.

DISCUSSION

In this study, we present two lines of evidence that suggest that the observed effects of peripheral administration of a synthetic peptide amide [LEP-(116–130)] on body weight and food intake in female *ob/ob* mice (15) may not be mediated by peptide activation of hypothalamic OB-R_b. In GT1–7 cells cotransfected with cDNA for OB-R_b and the reporter gene construct HRRE-SEAP, LEP-(116–130) was unable to induce SEAP activity. In addition, LEP-(116–130) was unable to inhibit binding of the AP-OB fusion protein to COS-7 cells expressing OB-R, even at concentrations as high as 300 µmol/l. Our in vitro data clearly indicate that activation of OB-R_b by LEP-(116–130) is not required for its effects on the energy balance in vivo.

That LEP-(116–130) was able to reduce body weight gain but not food intake in *db/db* mice that do not express OB-R_b provides further support for a mechanism of action different from that of leptin. The inability of LEP-(146–160) to induce similar changes indicates that the effect of LEP-(116–130) on body weight was not a generalized response to peptide treatment. We have not yet determined the mechanism by which peripheral administration of LEP-(116–130) reduces body weight gain in either *ob/ob* or *db/db* mice or whether the effects of LEP-(116–130) would have been more robust had it been administered centrally. We are actively investigating these questions.

We considered the possibility that the ability of LEP-(116–130) to reduce body weight gain in *db/db* mice may

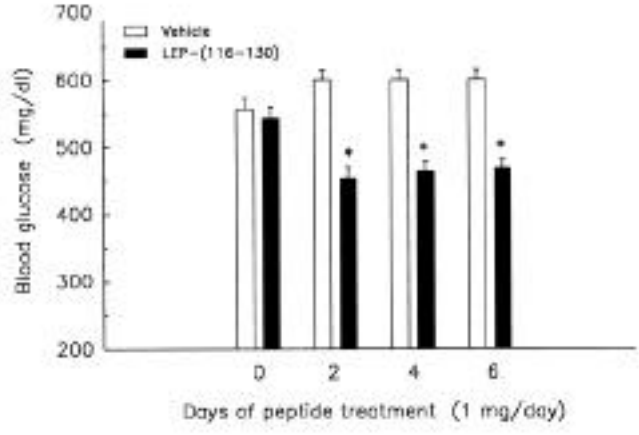

FIG. 4. Effects of LEP-(116–130) on blood glucose levels in female C57BLKS/6-m *db/db* mice. Mice were treated with vehicle or 1 mg i.p. LEP-(116–130) for 7 days. Blood was drawn from the tail vein at the beginning of the study (day 0) and after 2, 4, and 6 days of treatment. Each bar and vertical line represents the mean ± SE for glucose levels in a group of six mice. *Blood glucose level is significantly ($P < 0.05$) lower than in vehicle-injected control mice.

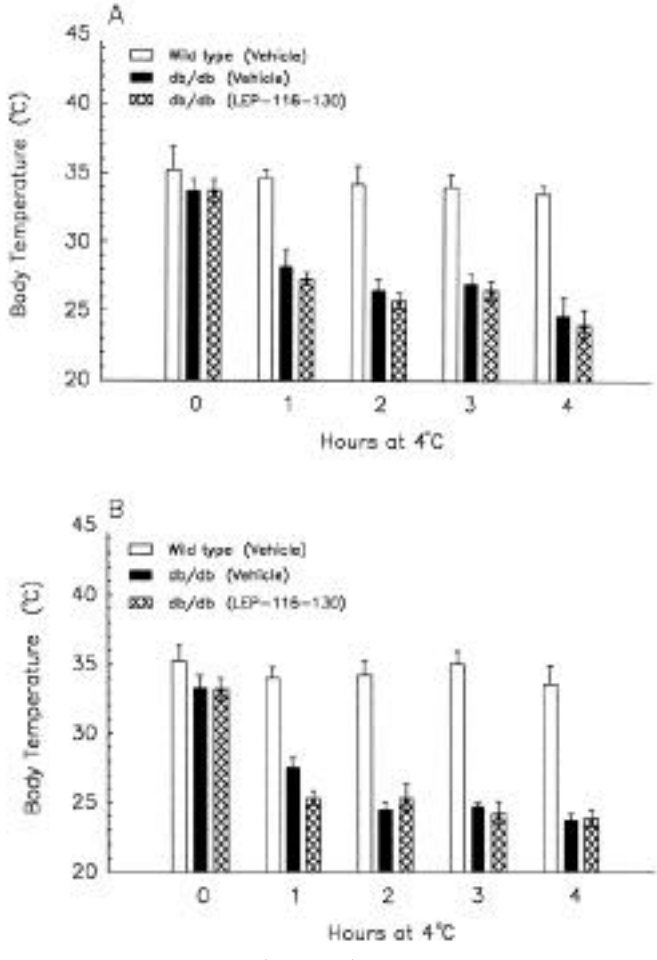

FIG. 5. Effects of LEP-(116–130) on thermogenesis in female C57BLKS/6-m *db/db* mice. Mice were treated with vehicle or 1 mg i.p. LEP-(116–130) for 7 days. On days 4 (*A*) and 7 (*B*), the mice were placed in a cold room (ambient temperature of 4°C). Body temperatures were measured with a rectal probe 1, 2, 3, and 4 h later. Each bar and vertical line represents the mean ± SE for core temperatures in a group of six mice.

TABLE 3
Effects of LEP-(116–130) on body weight gain in female wild-type C57BLKS/6-m mice after 7 days of treatment

	Vehicle	LEP-(116–130)
Initial weight (g)	17.7 ± 0.7	18.0 ± 0.9
Weight after 7 days (g)	18.3 ± 0.9	18.2 ± 1.0
Weight increase (g)	0.6	0.2
Increase (%)	3.4	1.1
*P**		0.047

Data are means ± SE or % (n = 6). *The mean of the differences between the initial and 7-day body weight of each mouse (n = 6) in the group treated with LEP-(116–130) was compared with the mean of the differences between the initial and 7-day body weight of each vehicle-injected control mouse (n = 6). The two-tailed P value was determined with the Wilcoxon signed-rank test.

TABLE 4
Cumulative effects of LEP-(116–130) (1 mg/day i.p.) on food intake in female wild-type C57BLKS/6-m mice after 7 days of treatment

Treatment	Cumulative food intake (g/mouse)
Vehicle	17.5 ± 0.7
LEP-(116–130)	19.5 ± 0.4
*P**	0.079

Data are means ± SE (n = 6). *The cumulative food intake by each mouse in the group treated with LEP-(116–130) (n = 6) was compared with the cumulative food intake of each vehicle-injected control mouse (n = 6). The two-tailed P value was determined with the Wilcoxon signed-rank test.

have been related to some undetermined toxic side effect of the peptide. Although we cannot completely eliminate this possibility, several lines of evidence suggest that this was not the case. First, we noted no negative effect of LEP-(116–130) on appetite in these mice. Their rate of food intake was similar to that of vehicle-injected control animals throughout the course of the study. Second, no observable ill effects of LEP-(116–130) were evident from the appearance or behavior of the mice. Their coat quality, stools, and activity level were similar to those of vehicle-injected control mice. Third, the inhibitory effects of LEP-(116–130) on body weight gain were apparent in the absence of any significant reduction in food intake. This suggests that mice receiving LEP-(116–130) may have been more metabolically active than vehicle-injected control mice, which would not be the case if LEP-(116–130) was in some way toxic.

To understand the mechanism by which LEP-(116–130) exerts its influence on energy balance in *db/db* mice, we examined its effects on blood glucose levels. The reduction in blood glucose observed in mice given LEP-(116–130) for 7 days versus vehicle-injected control mice cannot be attributed to reduced caloric intake because peptide-treated mice consumed ~15% more food than vehicle-injected control mice during the study. This observation suggests that, via metabolic mechanisms that may be similar to those of leptin in *ob/ob* mice (21), LEP-(116–130) may also stimulate glucose utilization and in this way help to modulate energy balance.

The apparent uncoupling effect of LEP-(116–130) on food intake and body weight gain in *db/db* mice is noteworthy. Contrary to what was seen in our earlier studies with *ob/ob* mice in which weight loss was accompanied by reduced food intake (15), *db/db* mice given LEP-(116–130) in the present study did not lose weight but failed to gain additional weight even when their food intake was greater than that of vehicle-injected control mice. Our previous observations suggested that LEP-(116–130) may act via a mechanism related to the regulation of thermogenesis. The present data argue against this hypothesis because the observed reduction in body weight gain in mice treated with LEP-(116–130) was not accompanied by any improvement in their ability to thermoregulate.

The effects of LEP-(116–130) on body weight gain and food intake in wild-type (+/+) mice mimicked those in *db/db* mice. In both cases, body weight gain was reduced approximately threefold in the presence of increased food intake. These observations suggest that the effect of LEP-(116–130) on body weight gain may be the result of undetermined metabolic effects that are not dependent on or restricted to the presence of the obese phenotype.

Contrary to its effect on blood glucose in the severely hyperglycemic *db/db* mouse, however, administration of LEP-(116–130) to wild-type (+/+) mice for 7 days neither elevated nor lowered normal blood glucose levels in this animal model, which suggests an action of LEP-(116–130) that is specific to the diabetic phenotype. Our data with female *ob/ob* mice show a similar ability of LEP-(116–130) (1 mg/day i.p. for 7 days) to lower blood glucose levels by ~100 mg/dl, which lends further support to the specificity of the antihyperglycemic action of LEP-(116–130).

In summary, by using both in vitro and in vivo bioassay systems, our data suggest that the antiobesity effects of LEP-(116–130) may not be mediated by activation of hypothalamic OB-R_b. We cannot completely eliminate the possibility that LEP-(116–130), which is unable to bind and activate OB-R in vitro, may be modified intravascularly in the *ob/ob* mouse in such a way that a conformation that enables OB-R_b activation is achieved. Such an event, however, cannot account for the effect of LEP-(116–130) on body weight gain in *db/db* mice that do not express OB-R_b.

Unlike recombinant leptin, LEP-(116–130) was effective in both leptin-deficient (*ob/ob*) and leptin-resistant (*db/db*) animal models of obesity. Because most obese humans are not leptin deficient (22) and have normal leptin receptors (23), this observation suggests that the development of more potent peptide analogs or nonpeptide mimetics of LEP-(116–130), which can augment the effects of endogenous leptin independent of hypothalamic OB-R_b activation, may have potential application to the treatment of human obesity and its related dysfunctions.

ACKNOWLEDGMENTS

This work was supported by a grant from the Dr. Willard B. and Lulah Warring Memorial Fund and by funding from Hoffman-LaRoche (Nutley, NJ).

The authors thank Marilyn Brown for excellent technical support.

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