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Journal

American Journal of Physiology, 274(5)

ISSN

0002-9513

Authors

Havel, Peter J
Uriu-Hare, Janet Y
Liu, Tina
[et al.](#)

Publication Date

1998-05-01

DOI

10.1152/ajpregu.1998.274.5.r1482

Peer reviewed

Marked and rapid decreases of circulating leptin in streptozotocin diabetic rats: reversal by insulin

PETER J. HAVEL, JANET Y. URIU-HARE, TINA LIU, KIMBER L. STANHOPE, JUDITH S. STERN, CARL L. KEEN, AND BO AHRÉN

Department of Nutrition, University of California, Davis, California 95616; and Department of Medicine, Lund University, S-20502 Malmö, Sweden

Havel, Peter J., Janet Y. Uriu-Hare, Tina Liu, Kimber L. Stanhope, Judith S. Stern, Carl L. Keen, and Bo Ahrén. Marked and rapid decreases of circulating leptin in streptozotocin diabetic rats: reversal by insulin. *Am. J. Physiol.* 274 (Regulatory Integrative Comp. Physiol. 43): R1482–R1491, 1998.—Evidence for regulation of circulating leptin by insulin is conflicting. Diabetes was induced in rats with streptozotocin (STZ; 40 mg·kg⁻¹·day⁻¹ × 2 days) to examine the effect of insulin-deficient diabetes and insulin treatment on circulating leptin. After 12 wk, plasma leptin concentrations in untreated rats were all <0.4 ng/ml versus 4.9 ± 0.9 ng/ml in control animals (*P* < 0.005). In rats treated with subcutaneous insulin implants for 12 wk, which reduced hyperglycemia by ~50%, plasma leptin was 2.1 ± 0.6 ng/ml, whereas leptin concentrations were 6.0 ± 1.6 ng/ml in insulin-implanted rats receiving supplemental injections of insulin for 4 days to normalize plasma glucose (*P* < 0.005 vs. STZ untreated). In a second experiment, plasma leptin was monitored at biweekly intervals during 12 wk of diabetes. In rats treated with insulin implants, plasma leptin concentrations were inversely proportional to glycemia (*r* = -0.64; *P* < 0.0001) and unrelated to body weight (*P* = 0.40). In a third experiment, plasma leptin concentrations were examined very early after the induction of diabetes. Within 24 h after STZ injection, plasma insulin decreased from 480 ± 30 to 130 ± 10 pM (*P* < 0.0001), plasma glucose increased from 7.0 ± 0.2 to 24.8 ± 0.5 mM, and plasma leptin decreased from 3.2 ± 0.2 to 1.2 ± 0.1 ng/ml (Δ = -63 ± 3%, *P* < 0.0001). In a subset of diabetic rats treated with insulin for 2 days, glucose decreased to 11.7 ± 3.9 mM and leptin increased from 0.5 ± 0.1 to 2.9 ± 0.6 ng/ml (*P* < 0.01) without an effect on epididymal fat weight. The change of leptin was correlated with the degree of glucose lowering (*r* = 0.75, *P* < 0.05). Thus insulin-deficient diabetes produces rapid and sustained decreases of leptin that are not solely dependent on weight loss, whereas insulin treatment reverses the hypoleptinemia. We hypothesize that decreased glucose transport into adipose tissue may contribute to decreased leptin production in insulin-deficient diabetes.

glucose; C-peptide; food intake; epididymal fat

LEPTIN, THE PRODUCT of the *ob* gene (48), has been implicated in the regulation of feeding behavior and body adiposity (7). Circulating leptin concentrations correlate with adiposity in both humans (10, 18, 29) and rodents (1, 29) and decrease after fasting (1, 3, 5, 46), energy restriction (13), or weight loss (10, 18). Thus leptin may act as a signal from peripheral adipose stores to the central nervous system to decrease food intake, increase energy expenditure, and limit adiposity (7). Conversely, low leptin concentrations after fasting or energy restriction may act to increase food intake and decrease energy expenditure (7) as well as

to regulate neuroendocrine and reproductive function (3). However, the factors involved in the physiological regulation of leptin secretion and circulating leptin concentrations are not well defined.

Plasma leptin concentrations are correlated with plasma insulin concentrations in humans and animals (1, 18) and are related to indexes of insulin secretion and negatively correlated with insulin sensitivity, independent of adiposity, in humans (26). Changes of plasma leptin after fasting in animals (1) or after weight loss in humans (18) are correlated with changes of plasma insulin independent of changes in adiposity. In addition, decreases of circulating leptin during energy restriction in human subjects are related to changes of insulin and glucose (13), and glucose infusions that prevent the decreases of insulin and glucose during fasting also prevent the decline of circulating leptin (5). Furthermore, nocturnal increases of plasma leptin concentrations in humans (40) are proportional to insulin responses to meals (27).

Thus, based on the relationship between circulating insulin and leptin concentrations, insulin is a candidate as a hormonal regulator of leptin synthesis and/or secretion. Although several studies investigating the effect of insulin administration on circulating leptin have found no changes of plasma leptin during short-term hyperinsulinemic euglycemia or hypoglycemic clamps (12, 23, 26, 34), in one study, long-term (72 h) insulin infusion modestly increased plasma leptin (23). Two other studies examined the effects of high-dose insulin and glucose infusion and found increased plasma leptin after 4–6 h in nondiabetic (45) and diabetic (19) human subjects. These infusions, however, produced markedly supraphysiological insulin concentrations and required the administration of large amounts of glucose to prevent hypoglycemia. Thus these studies did not address whether the increases of circulating leptin were due to a direct effect of insulin per se or an indirect effect via insulin's action to increase adipose glucose uptake. We have recently reported that glucose uptake and metabolism are involved in regulating leptin expression and secretion in cultured rat adipocytes (31).

Several studies have found that *ob* gene expression is increased after insulin (11, 16, 30, 35) or glucose (30) administration and decreased in insulin-deficient diabetic animals (4, 28, 42); however, changes of circulating leptin concentrations after induction of diabetes and plasma leptin responses to insulin administration in diabetic rodents have not been previously reported. Furthermore, dissociations between changes of *ob* gene expression and circulating leptin concentrations have recently been described (22), suggesting that leptin

expression and secretion can be differentially regulated.

Therefore, the goals of the present study were to examine the effects and time course of insulin-deficient diabetes induced by streptozotocin (STZ) on circulating leptin concentrations, the effects of short-term and long-term exogenous insulin treatment on plasma leptin, and the relationship of plasma leptin to changes of glycemia in STZ diabetic rats. STZ diabetes results in weight loss. Because loss of body fat would be expected to reduce circulating leptin concentrations, we also examined plasma leptin very early after the induction of diabetes, before the onset of significant weight loss.

METHODS

Animals

Adult male Sprague-Dawley rats (Charles River) weighing 340–490 g were used for the studies. The animals were individually housed in hanging wire cages and fed a standard commercial diet (no. 557; Ralston Purina, Belmont, CA) and deionized water ad libitum. The light-dark cycle was 12 h with lights on and 12 h with lights off, with lights on at 0600. Experimental protocols were approved by the Institutional Animal Use and Care Committee of the University of California, Davis and were conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*.

Induction of Diabetes

Insulin-deficient diabetes was induced with subcutaneous injections of freshly prepared STZ (Sigma, St. Louis, MO) at a dose of 40 mg/kg in ice-cold 0.5 mol/l citrate buffer (pH 4.5). A second dose of STZ (40 mg/kg) was administered 24 h later. This regimen produces insulin-deficient diabetes (plasma glucose >25 mmol/l) in >95% of treated animals without inducing renal failure or losses from hypoglycemia (2). Control animals received injections of citrate buffer only. Three separate experiments were conducted in STZ diabetic rats: two long-term 12-wk studies and one short-term 2-wk study.

Protocols

Experiment 1. To examine plasma leptin after long-term (12 wk) STZ diabetes and the effects of two levels of insulin treatment designed to either reduce or normalize plasma glucose concentrations on circulating leptin, diabetes was induced with STZ in 24 rats as described in *Induction of Diabetes*. Twelve buffer-injected rats served as nondiabetic controls. At the time of the second STZ injection, 14 of the diabetic animals received 1.25 implants impregnated with bovine insulin (Linplant, Toronto, ON, Canada) placed subcutaneously through a 14-gauge needle under light ketamine-xylazine (30:10 mg/kg, respectively) anesthesia. These implants slowly release insulin (~2 U/day) for up to 40 days. The control and 10 other STZ diabetic rats were sham implanted. Plasma glucose was monitored 1–2 times weekly from a tail blood sample (~0.5 ml) collected in a heparinized tube (Sarstedt, Hayward, CA) during brief restraint (<2 min) in a polyethylene rodent restraint cone. Plasma glucose was markedly elevated (>25 mmol/l) in all of the untreated STZ diabetic rats. The implanted animals were reimplanted with an additional 0.5–2 implants to maintain a moderate reduction of plasma glucose (~10 mmol/l less than untreated rats). The untreated STZ diabetic rats all received one-third of an

insulin implant at 6 wk after the induction of diabetes to prevent marked weight loss and debilitation. The animals were weighed weekly, and food intake was determined periodically during the experiment. After 11 wk, 7 of the 14 implanted animals received daily injections of bovine ultra lente insulin (9–26 U·kg⁻¹·day⁻¹) to normalize the plasma glucose over 7 days. After 12 wk, all animals were anesthetized with chloral hydrate (350 mg/kg) and a blood sample (3.0 ml) was obtained between 1000 and 1400 by cardiac puncture with a 21-gauge needle before the pancreas was isolated and perfused for another study.

Experiment 2. To examine plasma leptin over the course of long-term (12 wk) STZ diabetes and its relationship to glycemic control during insulin treatment, diabetes was induced in 29 rats. Eighteen buffer-injected rats served as nondiabetic controls. Plasma glucose was monitored weekly by tail bleeding as described in *experiment 1*. Seventeen of the diabetic rats received 1.25 subcutaneous insulin implants after the second STZ injection as described in *experiment 1*. After 3 wk, plasma glucose concentrations were increasing. Therefore, an additional one to two implants (mean = 1.5) were placed after 3 wk. Most of the animals (14 of 17) received an additional implant after 8 wk to maintain a moderate reduction of plasma glucose (see Fig. 2A). Twelve of the STZ diabetic rats were untreated except for 0.5 implants placed 6 wk after the induction of diabetes. The animals were weighed weekly, and food intake was measured over 2 days every 2 wk during the experiment. Blood samples (1.8 ml) for the measurement of plasma leptin were obtained from nonfasted animals between 1000 and 1200 by tail bleeding, at biweekly intervals over the 12 wk of the experiment, into heparinized tubes containing 30 µl of aprotinin (Sigma) solution per milliliter of whole blood collected. A total of 10.8 ml was obtained over the first 10 wk of the study. A final blood sample (2.0 ml) was collected between 1000 and 1400 by cardiac puncture after 12 wk as described for *experiment 1*.

Experiment 3. To examine plasma leptin concentrations and their relationship to other parameters very early after the induction of STZ diabetes, diabetes was induced in 22 rats as described in *Induction of Diabetes*. Fourteen buffer-injected animals served as nondiabetic controls. Body weight, food intake, and water intake were measured for 2 days before the induction of diabetes and for the 14-day duration of the experiment. A baseline blood sample (1.0 ml) for measurement of plasma glucose, insulin, and leptin was collected before the initial injection of STZ, and additional 1.0-ml samples were taken at 1, 2, 3, 5, 8, 11, and 14 days after STZ for a total of 8.0 ml of blood over 2 wk. All blood samples were taken in the nonfasted state between 0800 and 1000. A subset of diabetic animals ($n = 8$) received twice-daily injections of lente insulin at 0800 and 1800 on *days 14–16*. On *day 16*, eight of the control rats, seven untreated diabetic rats, and the eight insulin-treated STZ diabetic rats were anesthetized with halothane and a final blood sample (3.0 ml) was collected via cardiac puncture. The animals were euthanized, and the liver, kidneys, and epididymal fat depots were removed and weighed.

Assays and Data Analysis

Plasma glucose was measured by the glucose oxidase method with a glucose analyzer 2700 (Yellow Springs Instruments). Plasma nonesterified fatty acid (NEFA) concentrations were measured with a kit from Waco (Richmond, VA). Plasma insulin and C-peptide were measured by radioimmunoassays specific for rat insulin and C-peptide with reagents from Linco Research (St. Charles, MO). The lower limits of detection for the rat insulin and C-peptide assays are 0.1

ng/ml and 40 pM, respectively. Plasma leptin was measured with a radioimmunoassay for mouse leptin with reagents from Linco Research as previously described (1). Plasma leptin concentrations measured with this assay are proportional to those obtained with a newly developed assay specific for rat leptin (25). Measured leptin concentrations were highly correlated between the two assays ($r = 0.94$, $P < 0.0001$). Measurements of rat plasma leptin made with the mouse assay therefore provide reliable estimates of rat leptin concentrations. The intra- and interassay coefficients of variation for the mouse assay are 4.0 and 11.2%, respectively (1), with a lower limit of detection of 0.2 ng/ml. The antibody used in the assay does not cross-react with insulin, pro-insulin, glucagon, pancreatic polypeptide, or somatostatin.

Data are expressed as means \pm SE. Statistical analyses were performed using a software package (StatView for Macintosh; Abacus, Berkeley, CA). Means within a group were compared with a paired t -test. For comparisons of more than two groups, analysis of variance (ANOVA) was used with Dunnett's post test. Both simple and multiple linear regression analyses were performed.

RESULTS

Experiment 1

Long-term effects of STZ diabetes and insulin treatment on plasma leptin concentrations. Body weight, food intake, plasma insulin, and C-peptide values measured after 12 wk of diabetes in the four groups of animals are provided in Table 1. As expected, untreated STZ diabetic rats were markedly hyperglycemic. Plasma glucose in insulin-implanted rats was significantly reduced compared with untreated animals but still elevated compared with nondiabetic control rats (Fig. 1A). Plasma glucose was normalized to control levels in insulin-implanted rats receiving insulin injections (Fig. 1A). Plasma leptin was <0.5 ng/ml ($\sim 10\%$ of mean control values) in all untreated STZ diabetic rats. In insulin-implanted diabetic rats, plasma leptin concentrations were $42 \pm 13\%$ of those in control animals.

Table 1. Body weight, food intake, plasma insulin, and C-peptide after 12 wk of diabetes

	Control Nondiabetic ($n = 11$)	STZ Untreated ($n = 10$)	STZ Implanted ($n = 7$)	STZ Implanted + Insulin Injected ($n = 7$)
Body wt, g (initial)	404 \pm 6	401 \pm 4	401 \pm 4	398 \pm 7
Body wt, g (12 wk)	631 \pm 22	465 \pm 13 \ddagger	551 \pm 17*	615 \pm 25
Δ Body wt, g/ 12 wk	227 \pm 18	65 \pm 11 \ddagger	150 \pm 14 \ddagger	217 \pm 20
Food intake, g/day	32 \pm 1	65 \pm 4 \ddagger	38 \pm 2	ND
[Insulin], pmol/l	188 \pm 22	46 \pm 15*	1,021 \pm 305 \ddagger	1,080 \pm 451 \ddagger
[C-peptide], pmol/l	591 \pm 72	49 \pm 6 \ddagger	78 \pm 17 \ddagger	<40 \ddagger

Values are means \pm SE; n = no. of rats. Nondiabetic control, untreated streptozotocin (STZ) diabetic, insulin-implanted STZ diabetic, and insulin-implanted diabetic rats treated with daily insulin injections from week 11 to week 12 (experiment 1). Plasma glucose and leptin concentrations (brackets) from these animals are included in Fig. 1. ND, not done. * $P < 0.025$, $\ddagger P < 0.005$, $\ddagger P < 0.001$ vs. control.

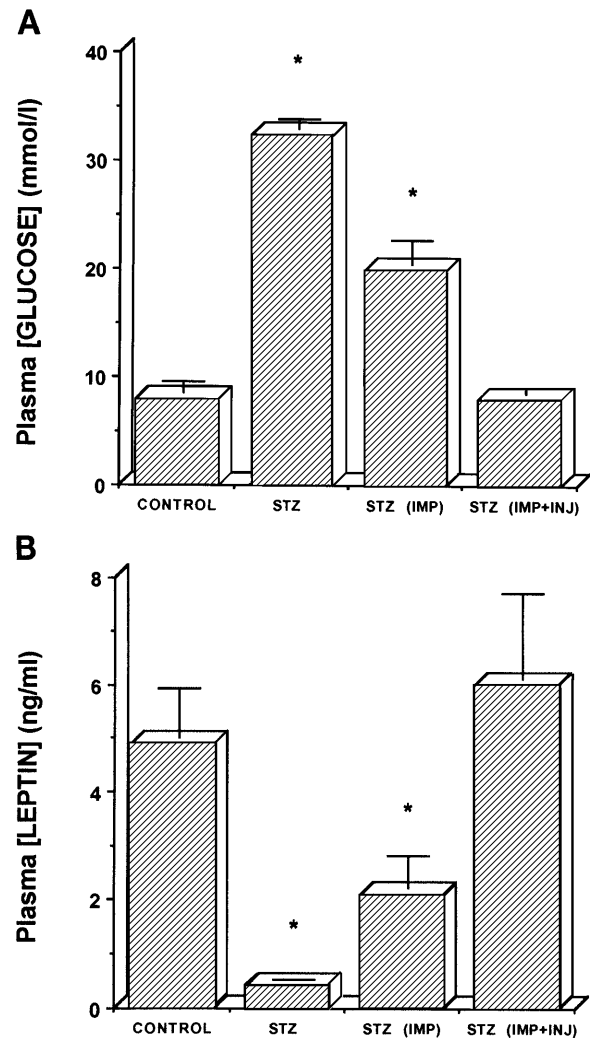


Fig. 1. Twelve-week plasma glucose (A) and leptin (B) concentrations in 12 nondiabetic (control) rats, 10 untreated streptozotocin (STZ) diabetic rats, 7 STZ diabetic rats with bovine insulin implants (Imp), and 7 insulin-implanted STZ diabetic rats also treated with insulin injections (Imp + Inj) to normalize plasma glucose concentrations. * $P < 0.025$ vs. control.

Plasma leptin was restored to levels equivalent to those in control animals ($122 \pm 34\%$ of control) in insulin-implanted animals receiving 7 days of supplemental insulin injections (Fig. 1B). Overall, plasma leptin after 12 wk was positively correlated with body weight ($r = 0.69$, $P < 0.0001$) and the change of body weight over 12 wk ($r = 0.71$, $P < 0.0001$) and negatively correlated with food intake ($r = -0.45$, $P < 0.01$), plasma glucose ($r = -0.60$, $P < 0.0002$), plasma insulin ($r = -0.49$, $P < 0.0025$), and plasma C-peptide ($r = -0.38$, $P < 0.025$).

Experiment 2

Time course of plasma leptin concentrations over 12 wk of insulin-treated STZ diabetes. In control rats, plasma glucose was stable at 7.1–8.3 mmol/l (Fig. 2A). The initial plasma leptin concentrations averaged 5.4 ± 0.5 ng/ml and remained between 5.1 and 7.5 ng/ml for the 12 wk of the experiment (Fig. 2B). Body weight in control rats was increased by 50 ± 3 g after 2 wk and

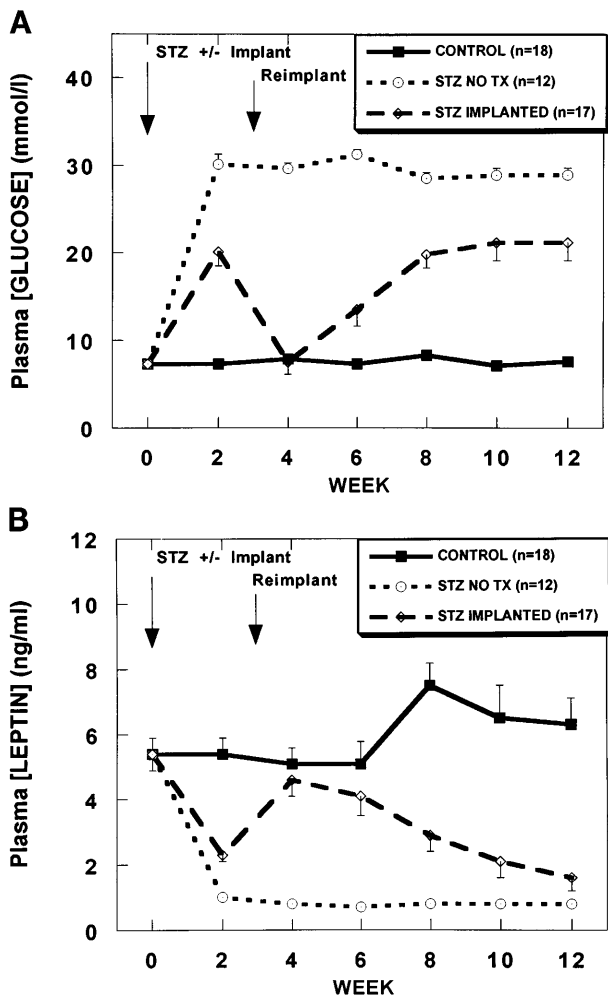


Fig. 2. Biweekly plasma glucose (A) and leptin (B) concentrations in nondiabetic (control) rats, untreated (no TX) STZ diabetic rats, and STZ diabetic rats treated with bovine insulin implants over 12 wk after STZ or vehicle injection.

continued to increase throughout the experiment, reaching 633 ± 12 g by 12 wk. In untreated diabetic rats, plasma glucose was increased to ~ 30 mmol/l at 2 wk after the induction of diabetes and remained elevated throughout the 12 wk of the experiment (Fig. 2A). Plasma leptin in untreated diabetic rats decreased to 1.0 ± 0.1 ng/ml by 2 wk ($P < 0.0001$ vs. control; Fig. 2B), whereas body weight increased by only 8 ± 3 g during this time ($P < 0.0001$ vs. control). In STZ diabetic rats with insulin implants, plasma glucose 2 wk after induction of diabetes was lower than in untreated diabetic rats (20.1 ± 1.6 mmol/l; Fig. 2A), and plasma leptin (Fig. 2B) and body weight gain were intermediate between control and untreated diabetic rats, averaging 2.3 ± 0.2 ng/ml and 24 ± 3 g, respectively.

After 3 wk, the insulin-implanted diabetic rats received an additional 1–2 insulin implants, which normalized plasma glucose concentrations at 7.5 ± 1.4 mmol/l at 4 wk (Fig. 2A). Plasma leptin increased to 4.6 ± 0.5 ng/ml at this time (Fig. 2B). Plasma glucose in this group of animals increased moderately at 6 wk and

more so from 8 to 12 wk into the study. Plasma leptin concentrations in insulin-implanted diabetic rats essentially mirrored plasma glucose concentrations throughout the study (Fig. 2, A and B). Body weight in insulin-implanted diabetic rats increased progressively but remained intermediate between the control and untreated diabetic animals.

Relationship between plasma leptin, plasma glucose, and body weight over 12 wk of STZ diabetes. Across all three groups of rats, mean plasma leptin concentrations over 12 wk were significantly correlated with mean body weight ($r = 0.62$, $P < 0.0001$) and negatively correlated with plasma glucose ($r = -0.78$, $P < 0.0001$) by simple linear regression. However, by multiple regression analysis, mean plasma leptin concentrations were significantly related to mean plasma glucose concentrations ($P < 0.0001$) but not to mean body weight ($P = 0.42$). When all six time points in the three groups of rats were examined, the individual plasma leptin concentrations were significantly correlated with both body weight ($r = 0.66$, $P < 0.0001$) and plasma glucose ($r = 0.74$, $P < 0.0001$) by simple and by multiple regression analysis ($r = 0.78$, $P < 0.0001$); however, the *t*-statistic was larger for plasma glucose ($t = 10.04$) than for body weight ($t = 6.19$). When these comparisons were made only in the group of STZ diabetic rats treated with insulin implants, plasma leptin concentrations over the six time points were negatively correlated with plasma glucose ($r = -0.64$, $P < 0.0001$; Fig. 3) but not body weight ($r = 0.14$, not significant) by simple regression analysis. Similarly, by multiple regression analysis, plasma leptin concentrations were related to plasma glucose concentrations ($P < 0.0001$) but not to body weight ($P = 0.40$).

Experiment 3

Short-term effects of STZ diabetes and insulin treatment on plasma leptin concentrations. BASELINE CORRELATIONS. Baseline characteristics of the animals studied in experiment 3 before the induction of diabetes are

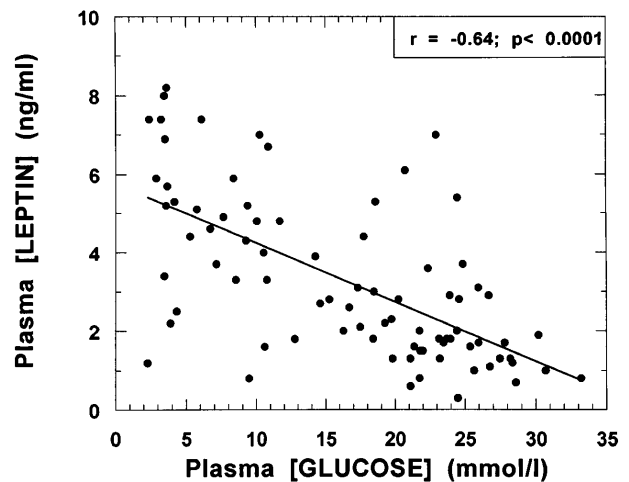


Fig. 3. Relationship between plasma glucose and leptin concentrations in 22 STZ diabetic rats treated with bovine insulin implants over the course of 12 wk of STZ diabetes. Plasma glucose varied widely in these animals over the 12 wk (see Fig. 2B).

included in Table 2. Baseline body weight in nonfasted animals was significantly correlated with plasma leptin concentrations ($r = 0.43$, $P < 0.01$) but not with plasma insulin ($r = 0.07$, $P = 0.71$) or plasma glucose concentrations ($r = 0.05$, $P = 0.79$). Baseline plasma leptin was significantly correlated with plasma insulin ($r = 0.46$, $P < 0.005$) but not with plasma glucose ($r = 0.13$, $P = 0.45$). Baseline plasma insulin was not correlated with baseline plasma glucose concentrations ($r = 0.06$, $P = 0.72$).

CHANGES AFTER THE INDUCTION OF DIABETES. Means of all parameters measured before and 14 days after the administration of STZ or vehicle (control animals) are provided in Table 2. The time courses of the changes in insulin, glucose, and leptin are shown in Figs. 4–6. Plasma insulin fell by 20% within 24 h of the first STZ injection ($P < 0.02$) and by $72 \pm 3\%$ ($P < 0.0001$) after 48 h and a second injection of STZ. Thereafter, plasma insulin remained at $<20\%$ of baseline concentrations (Fig. 4) despite marked hyperglycemia. Plasma glucose increased to 22.2 ± 1.3 mM 24 h after the first STZ injection ($P < 0.0001$) and continued to be elevated at >25 mM for the 14 days of the study (Fig. 5). Water intake increased progressively from the induction of diabetes throughout the 14 days of the experiment (Table 2). Body weight decreased slightly ($\sim 3\%$) 24 h after the first STZ injection and by 9% 24 h after the second injection.

Plasma leptin decreased $43 \pm 4\%$ ($P < 0.0001$) 24 h after STZ administration and by $63 \pm 3\%$ ($P < 0.0001$) after 48 h and a second STZ injection. At 72 h, plasma leptin was decreased by $73 \pm 2\%$ ($P < 0.0001$) and remained $<75\%$ of baseline concentrations for the 14 days of the study (Fig. 6). By simple linear regression, the change of plasma leptin at 24 h after STZ was significantly correlated with the changes of plasma glucose ($r = 0.69$, $P < 0.0005$), plasma insulin ($r = 0.58$,

Table 2. Body weight, food intake, H_2O intake, plasma insulin, glucose, and leptin before and after 14 days in nondiabetic rats and STZ diabetic rats (experiment 3)

	Control (n=14)	STZ Diabetic (n=22)
Body wt, g	433 \pm 9	438 \pm 7
Body wt (14 days), g	475 \pm 12	422 \pm 7*
Δ Body wt, g	+42 \pm 6*	-15 \pm 5 \dagger
Food intake, g/day	34 \pm 1	34 \pm 1
Food intake (14 days), g/day	34 \pm 1	70 \pm 1*
Δ Food intake, g/day	0 \pm 1	+36 \pm 1* \dagger
H_2O intake, g/day	48 \pm 3	47 \pm 3
H_2O intake (14 days), g/day	43 \pm 2	266 \pm 9*
ΔH_2O intake, g/day	-4 \pm 3	+219 \pm 9* \dagger
[Insulin], pmol/l	417 \pm 30	482 \pm 34
[Insulin] (8 days), pmol/l	365 \pm 46	84 \pm 10*
Δ [Insulin], pmol/l	-52 \pm 57	-398 \pm 30* \dagger
[Glucose], mmol/l	6.9 \pm 0.2	7.1 \pm 0.2
[Glucose] (14 days), mmol/l	7.1 \pm 0.2	27.6 \pm 0.7*
Δ [Glucose], mmol/l	+0.1 \pm 0.2	+20.6 \pm 0.8* \dagger
[Leptin], ng/ml	3.2 \pm 0.2	3.2 \pm 0.2
[Leptin] (14 days), ng/ml	3.1 \pm 0.3	0.6 \pm 0.1*
Δ [Leptin], ng/ml	-0.1 \pm 0.3	-2.7 \pm 0.2* \dagger

Values are means \pm SE; n = no. of rats. * $P < 0.0001$ vs. day 0, $\dagger P < 0.0001$ vs. control, $\ddagger P < 0.005$ vs. day 0.

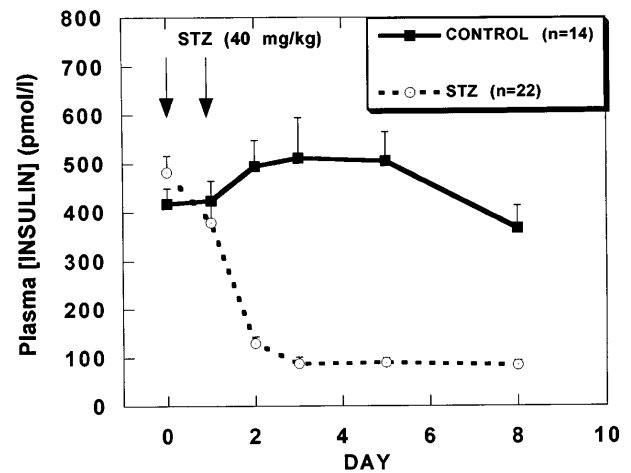


Fig. 4. Plasma insulin concentrations in nondiabetic (control) rats and untreated STZ diabetic rats before and after induction of diabetes. Arrows indicate injection times.

$P < 0.005$), and body weight ($r = 0.69$, $P < 0.0005$). However, by multiple regression, the change of leptin correlated with the change of glucose ($P = 0.018$) but not the change of insulin ($P = 0.14$) or the change of body weight ($P = 0.56$). Food intake was initially reduced by $\sim 20\%$ after STZ injection but recovered to that of control rats by day 3. STZ diabetic rats were hyperphagic relative to nondiabetic control animals by day 4. Food intake in the diabetic animals increased progressively thereafter and was approximately double that in control rats from days 8–14 (Table 2).

EFFECTS OF EXOGENOUS INSULIN ADMINISTRATION. A subset of eight STZ diabetic rats from experiment 3 was treated with insulin for 2 days from day 14 to day 16. Responses to insulin treatment are included in Table 3. Parameters examined in the insulin-treated STZ diabetic rats were compared with those in control and untreated diabetic rats after 16 days in Table 4. After 2 days of insulin injections, plasma glucose was reduced by $\sim 60\%$ and plasma leptin was increased by nearly 500%. Body weight was increased by $<3\%$. Food

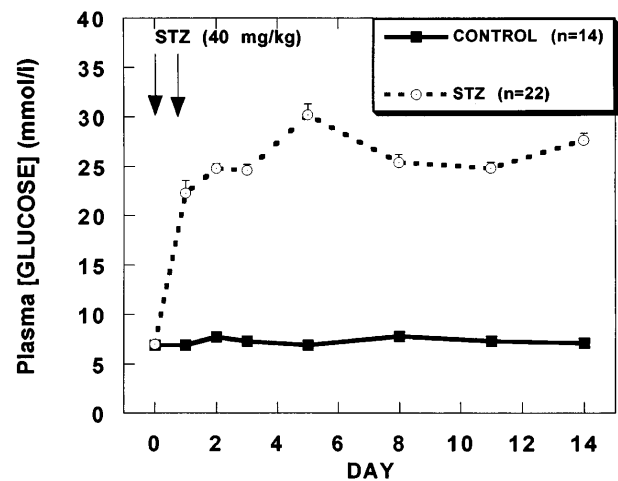


Fig. 5. Plasma glucose concentrations in nondiabetic (control) rats and untreated STZ diabetic rats before and after induction of diabetes. Arrows indicate injection times.

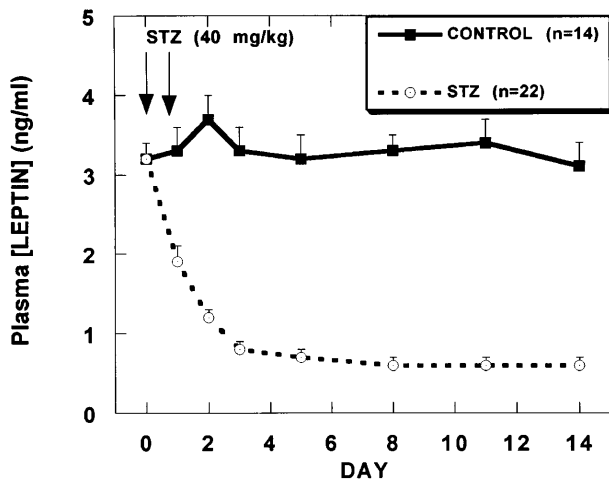


Fig. 6. Plasma leptin concentrations in nondiabetic (control) rats and untreated STZ diabetic rats before and after induction of diabetes. Arrows indicate injection times.

and water intake were significantly reduced by insulin treatment (Table 3). The increase of plasma leptin induced by insulin treatment was proportional to the degree of glucose lowering ($r = 0.75, P < 0.05$) and to liver weight ($r = 0.70, P < 0.05$) but not to the epididymal fat depot weight ($r = 0.12, P = 0.78$).

Plasma glucose concentrations in insulin-treated diabetic rats were significantly lower than in untreated diabetic rats and not different from the control rats. Plasma leptin in insulin-treated rats was increased compared with the untreated rats (Table 4). Plasma NEFA was elevated twofold in untreated diabetic rats and normalized in insulin-treated diabetic rats (Table 4). The insulin-treated rats weighed less than control rats and were not significantly heavier than untreated diabetic rats (Table 4). Epididymal fat depot weight was also significantly lower in insulin-treated than in control rats and not different from untreated diabetic rats. Insulin-treated diabetic rats had larger livers than control or untreated diabetic rats. Both insulin-treated and untreated diabetic rats had larger kidneys than nondiabetic control animals (Table 4). Insulin-treated diabetic rats had lower food intakes compared with untreated diabetic rats but were still hyperphagic relative to the control animals.

In all 23 animals studied on day 16, plasma leptin concentrations were significantly correlated with both plasma glucose ($r = 0.76, P < 0.0001$) and plasma NEFA ($r = 0.60, P < 0.005$). In addition, plasma glucose

Table 3. Values before and after 2 days of insulin administration in 8 STZ diabetic rats (experiment 3)

	Before Insulin	After Insulin	Δ
[Insulin], pmol/l	90 ± 0.20	5,650 ± 2,050	+5,560 ± 2,060*
[Glucose], mmol/l	27.9 ± 1.3	11.7 ± 3.9	-16.2 ± 4.7†
[Leptin], ng/ml	0.5 ± 0.1	2.9 ± 0.6	+2.4 ± 0.7†
Body wt, g	426 ± 11	437 ± 10	+11 ± 4*
Food intake, g/day	74 ± 2	59 ± 2	-16 ± 3‡
H ₂ O intake, ml/day	311 ± 18	132 ± 10	-179 ± 15‡

Values are means ± SE. * $P < 0.02$ vs. before insulin, † $P < 0.01$ vs. before insulin, ‡ $P < 0.0025$ vs. before insulin.

Table 4. Body weight; plasma glucose, NEFA, and leptin; liver, kidney, and epididymal fat depot weights; and food intake in rats after 16 days (experiment 3)

	Control Nondiabetic (n=8)	STZ Untreated (n=7)	STZ Insulin-Treated (n=8)
Body wt, g	489 ± 21	414 ± 6*	437 ± 10†
[Insulin], pmol/l	610 ± 70	80 ± 10*	5,560 ± 2,060†
[Glucose], mmol/l	9.4 ± 0.6	29.9 ± 1.2*	11.7 ± 3.9‡
[NEFA], meq/l	0.39 ± 0.03	0.85 ± 0.09*	0.34 ± 0.08‡
[Leptin], ng/ml	4.7 ± 0.6	0.6 ± 0.1*	2.9 ± 0.6§
Liver wt, g	17.3 ± 1.0	18.1 ± 0.6	27.0 ± 2.3*†
Kidney wt, g	3.7 ± 0.2	4.7 ± 0.1*	4.7 ± 0.3†
Fat wt, g	7.5 ± 0.7	3.1 ± 0.2*	4.4 ± 0.3*
Food intake, g/day	36 ± 2	74 ± 4*	58 ± 2*†

Values are means ± SE; n = no. of rats. STZ insulin-treated rats were injected with insulin from days 14–16. NEFA, nonesterified fatty acids. * $P < 0.005$ vs. control, † $P < 0.025$ vs. control, ‡ $P < 0.005$ vs. STZ untreated, and § $P < 0.025$ vs. STZ untreated.

and plasma NEFA concentrations were highly correlated with each other ($r = 0.82, P < 0.0001$). By multiple regression analysis, however, plasma leptin was correlated with plasma glucose ($P < 0.005$) but not with plasma NEFA ($P = 0.71$). Similarly, in these animals, plasma leptin concentrations were significantly correlated with both plasma glucose ($r = 0.76, P < 0.0001$) and epididymal fat depot weight ($r = 0.83, P < 0.0001$) by simple linear regression (Fig. 7, A and B). By multiple regression analysis, plasma leptin was significantly correlated with both epididymal fat depot weight and plasma glucose concentrations (both $P < 0.001$).

DISCUSSION

In this study, we found that circulating plasma leptin concentrations are markedly decreased in untreated STZ diabetic rats. Thus the effects of insulin-deficient diabetes on plasma leptin concentrations are in agreement with previous studies that showed reductions of adipose tissue *ob* mRNA in STZ diabetic rats (4, 28, 42). Plasma leptin was markedly decreased after 2 wk and remained low through 12 wk of STZ diabetes in rats with significantly lower body weights than in nondiabetic control rats (experiments 1 and 2). However, marked decreases of plasma leptin were also observed very early (24–48 h) after the induction of diabetes (experiment 3) and before major decreases of body weight. These early changes of leptin were proportional to the changes of glycemia but not to changes of body weight. Furthermore, it is likely that the weight loss during the first 24–48 h after induction of diabetes was mostly due to loss of body water rather than body fat, because diabetic rats were hyperglycemic and polydipsic by 24 h. Thus plasma leptin was decreased out of proportion to the modest changes of body weight.

Plasma leptin is similarly decreased during fasting (5, 46) or energy restriction in humans (13) and rodents (1, 3) to a degree beyond that expected for the decreases of body weight or fat content. Therefore, despite the close relationship between circulating leptin concentrations and adiposity, both short-term (24–48 h) diabetes

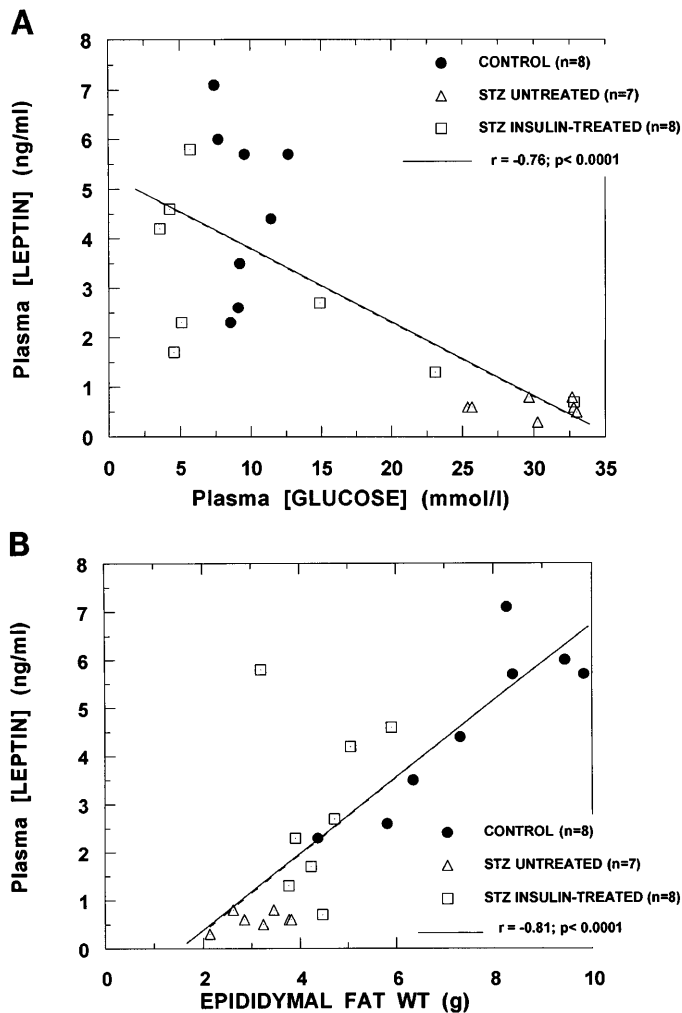


Fig. 7. Relationship between plasma glucose (A) or epididymal fat depot weight (B) and plasma leptin concentrations after 16 days in nondiabetic control rats, untreated STZ diabetic rats, and STZ diabetic rats treated with twice-daily insulin injection for 2 days after 14 days of diabetes.

and energy restriction are characterized by lower circulating leptin concentrations than expected for the amount of body fat. After 2 wk of diabetes, decreases of plasma leptin were related to reduced adiposity, as assessed by epididymal fat depot weight, and inversely correlated with plasma glucose concentrations. Together these data suggest that the short-term effect of STZ-induced diabetes to decrease plasma leptin is mainly the result of insulin deficiency and decreased glucose uptake and metabolism (see below), whereas in diabetes of longer duration (>2 wk), both reduced adiposity and insulin deficiency contribute to the hypo-leptinemia.

The relationship between adiposity, glycemia, and circulating leptin concentrations may vary with the time of day and the metabolic state. Sinha et al. (40) have shown that there is a nocturnal increase of plasma leptin concentrations in humans. The nocturnal increase does not occur in fasted subjects (5) and appears to be related to meal-induced insulin secretion and diurnal cortisol rhythms (27). A nocturnal increase of

ob gene expression has been observed in rats (35). Because we only measured leptin in nonfasted animals and at one time point during a single day, it is possible that the correlations we observed might be different if examined at other times. For example, circulating leptin concentrations may be most closely related to adiposity in the fasted state but related to plasma insulin, circulating glucose concentrations, and glucose metabolism in the fed state. We have, however, observed that in humans the relationship between fasting leptin and serum glucose is strengthened after 7 days of energy restriction and that the change of fasting leptin after energy restriction is correlated with changes of glycemia (13), suggesting that glucose metabolism may be related to leptin both after feeding and during fasting.

Either chronic (*experiments 1 and 2*) or acute (*experiment 3*) insulin treatment increased plasma leptin in STZ diabetic rats in proportion to the reduction of hyperglycemia. Insulin treatment has been previously demonstrated to increase *ob* mRNA in nondiabetic (11, 16, 35) rats and normalize *ob* mRNA expression in some (28), but not all (4, 42), studies of STZ diabetic rats. Dissociations between changes of *ob* gene expression and changes of circulating leptin concentrations have been reported. For example, plasma leptin increases after dexamethasone administration in humans without a corresponding increase of adipose *ob* mRNA (22). It should be noted that the circulating insulin concentrations produced by administering exogenous insulin to diabetic rats in these studies were substantially higher than in nondiabetic control animals. These levels are, however, necessary to lower plasma glucose concentrations because STZ diabetes is associated with insulin resistance (32) in addition to insulin deficiency.

A number of published studies have found that short-term insulin administration does not increase plasma leptin concentrations in human subjects (12, 23, 26, 34). In contrast, some other studies have demonstrated significant increases of circulating leptin concentrations after 4–6 h of high-dose insulin and glucose infusion in nondiabetic (45) and diabetic human subjects (19). Furthermore, infusions of glucose alone, resulting in hyperglycemia and concomitant endogenous hyperinsulinemia, increase plasma leptin within 4 h in humans (43) and rhesus monkeys (17).

There is evidence that the induction of marked hyperinsulinemia is not required to increase plasma leptin concentrations. For example, low-dose glucose infusion sufficient to prevent the decline of plasma insulin and glucose during fasting also prevents the decline of plasma leptin (5). In addition, lowering plasma glucose concentrations to euglycemia in hyperglycemic insulin-dependent diabetic human subjects by infusing insulin at rates that produced physiological insulinemia increases circulating leptin (20). Therefore, an effect of insulin to increase adipose tissue glucose uptake and metabolism, rather than hyperinsulinemia per se, may be involved in stimulating leptin secretion. Consistent with this hypothesis, we have found that stimulation of leptin secretion by insulin

from in vitro cultured rat adipocytes is closely related to the effects of insulin to increase adipocyte glucose uptake. In the same study, blocking glucose uptake with 2-deoxy-D-glucose, phloretin, or cytochalasin-B or inhibiting glucose metabolism with iodoacetate or sodium fluoride produced inhibitions of leptin secretion that were related to decreased glucose utilization, despite the presence of high insulin concentrations (31). Thus hypoleptinemia in unregulated insulin-deficient diabetes may be a consequence of decreased glucose uptake and metabolism in adipose tissue, whereas the restoration of circulating leptin levels by insulin treatment in diabetic animals may be secondary to increased adipocyte glucose uptake and utilization. We have hypothesized that similar, but less marked, changes of adipocyte glucose metabolism may mediate the in vivo effects of fasting and refeeding on circulating leptin concentrations (18).

Rats with uncontrolled diabetes in this study with low plasma leptin concentrations were markedly hyperphagic. Insulin treatment increased plasma leptin and reduced food intake. Schwartz et al. (37) have hypothesized that insulin acts as a long-term signal to the brain to regulate food intake. According to this model, hypoinsulinemia resulting in reduced insulin delivery to the central nervous system would contribute to diabetic hyperphagia. In one study, these investigators demonstrated that administration of insulin into the third ventricle of STZ diabetic rats at doses that did not alter peripheral insulinemia or plasma glucose levels inhibited the diabetic hyperphagia by ~50% and decreased, but did not normalize, the overexpression of neuropeptide Y (NPY) in the arcuate nucleus of the hypothalamus (41). Hypothalamic NPY, a potent stimulator of food intake (9), is upregulated in STZ diabetes (24, 41, 47). Because central insulin administration did not normalize food intake or NPY expression (41), another factor in addition to hypoinsulinemia, possibly decreased leptin, is likely to contribute to the hyperphagia.

Leptin deficiency results in hyperphagia (6, 15, 33, 36, 44), and leptin administration decreases food intake in rodents (6, 15, 33, 36, 44). Furthermore, hypothalamic NPY expression is increased in leptin-deficient (36, 39, 44) or leptin-resistant (8, 38) animals and is decreased by leptin administration in leptin-deficient or normal animals (36, 39, 44). Therefore, low circulating leptin concentrations may contribute, along with hypoinsulinemia, to hyperphagia in unregulated diabetes, perhaps via upregulation of the hypothalamic NPY system. In the current study, low leptin concentrations precede the onset of hyperphagia by several days. Therefore, if hypoleptinemia does contribute to diabetic hyperphagia, it is unlikely to be a fast-acting regulator. Experiments with leptin administration in diabetic animals will be necessary to test this hypothesis.

In summary, circulating leptin decreases markedly and rapidly after the induction of STZ diabetes in rats. Plasma leptin is restored by insulin administration in proportion to the degree of glucose lowering. Reductions of circulating leptin in unregulated diabetes may

be a consequence of decreased adipocyte glucose uptake and metabolism.

Perspectives

The present study, along with several previously published experiments, provides evidence that insulin and glucose are involved in regulating leptin production and secretion. We have reported recently that the uptake and utilization of glucose are key mediators of insulin-stimulated leptin expression and secretion from isolated adipocytes (31). Accordingly, changes of adipocyte glucose metabolism may be involved in effects of fasting and refeeding on circulating leptin concentrations in vivo. Similarly, increased adipocyte glucose metabolism resulting from insulin and glycemic responses to meals may contribute to the nocturnal rise of leptin concentrations in humans and animals. The biochemical mechanisms by which the metabolism of glucose in adipose tissue may be linked to leptin production are, however, not known. Although studies investigating the actions of leptin have been almost exclusively conducted in rodents, there is increasing evidence that leptin may have a role in regulating energy balance in humans (see Ref. 18). If this is the case, a detailed understanding of the mechanisms regulating leptin secretion may lead to new approaches for treating obesity by augmenting endogenous leptin production.

We acknowledge the expert technical assistance of Debbie Porter and thank Dr. Michael Schwartz for helpful advice and discussion of the results.

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-50129, DK-18899, DK-07355, and DK-35747, National Cancer Institute Grant CA-61654, the Swedish Medical Research Council (MX-6834), and the Juvenile Diabetes Foundation.

Address for reprint requests: P. J. Havel, Dept. of Nutrition, Univ. of California, Davis, One Shields Ave., Davis, CA 95616.

Received 3 December 1997; accepted in final form 11 February 1998.

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