Plasma Leptin in Diabetic and Insulin-Treated Diabetic and Normal Rats

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Adipose tissue leptin mRNA levels are decreased by food deprivation or induction of insulin-deficient diabetes. To determine whether plasma leptin concentrations are similarly affected, whether treatment of diabetes with insulin restores plasma leptin, and whether this requires restoration of body weight (lost as a result of diabetes) and/or normalization of glycemia, we measured plasma leptin concentrations in control, untreated streptozotocin (STZ)-diabetic, and insulin-treated STZ-diabetic rats. Plasma leptin was markedly reduced in untreated STZ-diabetic rats. Insulin treatment for 4 to 17 days increased plasma leptin approximately twofold above control levels. However, despite the hyperleptinemia, insulin-treated diabetic rats gained weight at a rate equal to that of sham-treated controls. Epididymal adipose tissue leptin mRNA levels in 17-day insulin-treated diabetic rats were equal to but did not exceed sham-control levels, unlike plasma leptin. Plasma glucose concentrations in insulin-treated STZ-diabetic rats were lower than in sham controls. Therefore, to determine whether hypoglycemia may be important in increasing plasma leptin, we measured plasma leptin levels in diabetic rats infused with insulin for 3 hours along with a variable-rate glucose infusion targeting glycemia to 200 or 40 mg/100 mL. Plasma leptin rapidly increased in these rats irrespective of target glycemia. Plasma leptin also increased rapidly in normal rats infused with insulin and glucose (target glycemia, 200 mg/100 mL). We conclude that plasma leptin concentrations are markedly reduced under conditions of insulin deficiency and rapidly increased by insulin treatment. The increase in plasma leptin does not require restoration of body weight and, under glucose clamp conditions, does not depend on target glycemia. Hyperleptinemia in insulin-treated diabetic rats is not explained on the basis of steady-state leptin mRNA levels, at least as reflected in epididymal fat.

LEPTIN, the *Lep* gene product, is an adipose cell–specific secreted protein postulated to provide a signal to the hypothalamus regarding the mass of energy stores in fat tissue.[1-3] Leptin appears to act through central (hypothalamic) regulatory centers to decrease appetite and increase energy utilization and to mediate some neuroendocrine responses to caloric deprivation. Consistent with this concept, our group and others have previously reported that adipose tissue leptin mRNA expression is reduced in insulin-deficient states associated with reduced fat mass including streptozotocin (STZ)-induced diabetes[4-6] and fasting.[4-12] Treatment of STZ-diabetic animals with insulin restores adipose tissue leptin mRNA expression.[4-6] However, this restoration is incomplete when body weight lost as a result of diabetes remains below baseline or prediabetic levels.[4,6]

STZ-diabetes with sufficient STZ dosing results in marked insulin deficiency. Treatment of STZ-diabetic rats by peripheral administration of human insulin results in hyperinsulinemia relative to levels in control rats and rapid improvement in glycemia to near-normal or subnormal concentrations depending on the insulin dosage and means of delivery (subcutaneous or intravenous in the studies reported here). We used the STZ-diabetes model to address the following additional questions: (1) Is plasma leptin concentration reduced in insulin-deficient diabetes (similar to leptin mRNA)?; (2) Is plasma leptin restored by insulin treatment?; and (3) Are recovery of body weight (lost as a result of diabetes) and/or normalization of glycemia necessary to restore plasma leptin or is insulin treatment sufficient in itself?

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MATERIALS AND METHODS

Animal Procedures

Male Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, IN) were fed standard rat chow and housed according to National Institutes of Health guidelines. All animals were allowed free access to food and water at all times. Two types of studies were performed. Group I studies examined the effects of STZ-diabetes and treatment for various periods with subcutaneous insulin. Group II studies consisted of a series of experiments in diabetic and nondiabetic rats treated with intravenous insulin and glucose.

Group I (effects of subcutaneous insulin on plasma leptin)

Twenty-one male Sprague-Dawley rats (age, 10 to 11 weeks; weight, 274 ± 2 g, mean ± SEM) were injected with STZ (125 mg/kg) intraperitoneally on day −2. Diabetes was diagnosed 2 days later (day 0), defined as a blood glucose greater than 300 mg/100 mL measured using an Accu-chek meter (Boehringer Mannheim, Indianapolis, IN) on 1 drop of blood obtained by tail vein puncture. Diabetic rats were treated with human regular (R) and NPH (N) insulin (2 U R at diagnosis of diabetes, and then 1R/3N at 6 PM and 1R/1N at 9 AM daily) for 2, 4, 10, or 17 days (days +2, +4, etc.) before death. Four control rats (age, 10 to 11 weeks; weight, 274 ± 1 g) were killed on day −2, and five control rats (initial weight, 272 ± 3 g) were treated with sham-STZ and sham-insulin injections and killed on day 17. Characteristics of these animals are shown in Table 1.

All animals were killed between 11 AM and 3 PM after food was removed at 8 AM. On the day of death, rats were anesthetized by methoxyflurane inhalation, and approximately 5 mL blood was immediately obtained by cardiac aspiration and placed in a heparinized tube. The epididymal fat pads were then dissected free, rinsed, blotted, weighed, quickly frozen in liquid nitrogen, and stored at −70°C. The heparinized blood was centrifuged at 2,000 × *g* for 5 minutes, and the plasma was divided into aliquots and frozen at −70°C until analysis.

Table 1. Alterations in Body Weight, Glucose, and Insulin in Group I Control, Diabetic, and Insulin-Treated Diabetic Rats

Group (day of death)	Initial Weight Day −2 (g)	Weight Loss in Diabetic Rats Day 0 (g)	Gain/Loss at Death (g)*	Glucose (mg/100 mL)	Insulin (ng/mL)	No. of Animals
C (d −2)	274 ± 1		0	167 ± 26	0.64 ± .18	4
D (d 0)	275 ± 4	−40 ± 3	−40 ± 3	505 ± 12	0.03 ± .03	4
Ins (d 2)	275 ± 3	−37 ± 2	−17 ± 10	262 ± 111	3.12 ± .71	4
Ins (d 4)	276 ± 4	−30 ± 1	−13 ± 14	94 ± 34	4.47 ± .25	4
Ins (d 10)	271 ± 1	−37 ± 3	22 ± 8	77 ± 9	3.20 ± .08	4
Ins (d 17)	275 ± 4	−38 ± 2	41 ± 4	155 ± 94	5.78 ± .51	5
C (d 17)	272 ± 3		90 ± 5	165 ± 4	1.96 ± .81	5

NOTE. STZ was administered on day −2, and diabetes was diagnosed on day 0. Insulin therapy was continued for 2 to 17 days as indicated. Four control rats were killed on day −2, and 5 control rats were treated with sham-STZ and sham-insulin and killed on day 17. Glucose and insulin were determined on blood obtained by cardiac puncture at the time of death. Data represent the mean ± SEM.

Abbreviations: C, control; D, diabetic; Ins, insulin-treated diabetic.

*Weight on day of death minus initial weight.

Group II (acute insulin/glucose infusion studies)

Diabetic rats. Male Sprague-Dawley rats (approximately 16 weeks of age) were injected with STZ (125 mg/kg) intraperitoneally. Diabetes was diagnosed 2 days later, and insulin was administered as in group I. Subsequently, the rats were maintained on insulin therapy as in group I for 2 to 16 days until the time of study. Insulin therapy was withheld on the evening before and the morning of the day of study. Food was removed from all rats at 7:30 AM, 3 to 4 hours before time 0 of the study periods. Characteristics of these animals are shown in Table 2.

Rats were prepared as previously described.[13] At 9 to 10 AM, rats were anesthetized with intraperitoneal methohexital sodium (40 mg/kg) and a catheter was inserted into the right femoral vein for maintenance of anesthesia with intravenous chloralose (50 mg/kg initially, and then 25 mg/kg/h). To prevent upper-respiratory tract obstruction and hypoxia, the trachea was cannulated for spontaneous respiration of O_2-enriched air. Sodium bicarbonate (0.1 mmol) was administered intravenously every 60 minutes. The rectal temperature was monitored continuously and maintained at 37.5°C using a heated surgical table and lamps. PE-50 catheters were inserted into the left femoral vein for infusion of insulin, the left femoral artery for continuous arterial pressure measurement and blood sampling, and the left jugular vein for infusion of glucose.

A continuous infusion of human R insulin (Lilly, Indianapolis, IN) was initiated and maintained at 1.0 U/h using a dual-roller pump (Biorad, Hercules, CA) to control flow. Whole blood glucose was maintained at either 200 or 40 mg/100 mL using a variable-rate infusion of 25% dextrose controlled by a Rainen (Woburn, MA) peristaltic pump and tubing with ID 0.02 mm calibrated to deliver a range of glucose infusion rates. Blood was sampled every 15 minutes by withdrawal of 30 μL whole blood from the jugular catheter, and glucose was determined using a Yellow Springs Instruments (YSI) analyzer (Yellow Springs, OH). To assist in maintaining target glycemia, blood glucose was also sampled every 5 minutes on 1 drop of blood using a reagent strip and meter calibrated previously to approximate the YSI readings. One thousand microliters of whole blood was removed at time 0, 1, 2, and 3 hours for measurement of plasma leptin and insulin levels. Centrifuged blood cells from these samples were returned to the rats.

Table 2. Characteristics of Group II Diabetic Rats

Parameter	Glycemia Target		
	200 mg/100 mL	40 mg/100 mL	Sham
Initial weight day −2 (g)	424 ± 2*	388 ± 7	391 ± 6
Weight loss day 0 (g)	−51 ± 2	−57 ± 3	−47 ± 4
Weight at death (g)	383 ± 2	352 ± 14	355 ± 6
Days treated†	8.1 ± 2.0	7.4 ± 1.1	6.2 ± 1.9
Insulin during infusion (ng/mL)‡			
Mean	99 ± 25	182 ± 41	0.12 ± 0.05
Range	38-147	48-291	0.03-0.36
No. of rats	7	7	6

NOTE. After induction of diabetes with STZ (day −2), diagnosis of diabetes (day 0), and subcutaneous insulin therapy for the number of days indicated, rats were subjected to acute infusion of insulin (1.0 U/h) and a variable-rate glucose infusion targeted to glycemia of 200 or 40 mg/100 mL. Subcutaneous insulin was withheld on the evening before and the morning of study. Data represent the mean ± SEM unless otherwise indicated.

*$P < .05$ by ANOVA compared with the 2 other groups.

†Days of subcutaneous insulin treatment (day 0 to time of study).

‡Average of means for each rat at 1, 2, and 3 hours of infusion.

To exclude nonspecific effects (unrelated to insulin or glucose) of these procedures on plasma leptin, we exposed additional STZ-diabetic rats to the same procedures described earlier and sham infusion of saline equal to the average volumes of glucose and insulin used in the experiments.

Non-diabetic rats. Nondiabetic, normal Sprague-Dawley rats (approximately 16 weeks of age) were studied in the same fashion using the same insulin infusion rate and target glycemia of 200 mg/100 mL. In addition, normal rats (approximately 16 weeks of age) underwent a sham procedure including administration of an equivalent volume of saline rather than insulin and glucose.

Plasma Assays

Plasma leptin levels were initially measured in group I rats by an enzyme-linked immunosorbent assay kindly performed by Dr Margery Nicolson (Amgen, Thousand Oaks, CA) using antibody to mouse leptin. All samples were tested in a single assay (one assay for each group). Recombinant rat leptin standards were used and showed parallel competition to mouse leptin. The interassay coefficient of variation (CV) was 5% and assay sensitivity 0.09 ng/mL. These samples, along with group II samples, were subsequently assayed for rat leptin using a kit purchased from Linco (St Louis, MO) that uses guinea pig polyclonal antibody to rat leptin. The interassay CV in our hands is 9% at 1.77 ng/mL and 12% at 6.27 ng/mL over four assays, and the assay range is 0.5 to 50 ng/mL. The results reported here were obtained using the Linco assay. Essentially the same relative results were obtained using the Amgen assay, although rat leptin values were lower by approximately 40%. Insulin was determined by radioimmunoassay using a kit also purchased from Linco. The interassay CV in our hands is 2% at 0.5 ng/mL, and the assay range is 0.1 to 10 ng/mL. Plasma glucose levels were measured using the YSI analyzer.

mRNA Measurement

Total RNA was extracted from epididymal adipose tissue as we have previously described.[14] Frozen tissue was thawed in cold guanidine isothiocyanate buffer homogenized using a polytron probe (Tekmar, Cincinnati, OH), and mRNA was prepared by centrifugation through $CsCl_2$. Specific mRNA levels within epididymal adipose total RNA were determined by Northern blot analysis using ^{32}P-random prime–labeled linearized cDNA probes. The full-length rat *Lep* cDNA was cloned and sequenced by polymerase chain reaction (PCR) as we previously described.[4] Full-length rat GLUT-4 cDNA[15] was a kind gift from Dr Morris Birnbaum at Harvard Medical School, and a 619-bp fragment of rat adipsin (nucleotides 73 to 701) was synthesized by PCR using the oligonucleotide primers 5′ ATTCTGGGTGGCCAGGAGGCCAT 3′ and 5′ TTTCTCCGGTTGCCACAGACT 3′.

Total cellular RNA (15 μg/lane) was size-fractionated on a 1% agarose/formaldehyde gel and transferred to nylon using a Hoefer Transphor apparatus (Hoefer Scientific, San Francisco, CA). Equivalent loading was verified by the density of the 28S and 18S ribosomal bands after transfer. Blots were prehybridized for 4 hours in 10% dextran sulfate, 40% deionized formamide, 4X SSC (1X SSC is 0.15 mol/L NaCl and 0.015 mol/L sodium citrate), 5X Denhardt solution (1 mg/ml ficoll, 1 mg/mL polyvinylpyrrolidine, and 1 mg/mL bovine serum albumin), 0.10 mg/mL sonicated /denatured salmon testes DNA, and 0.2 mol/L Tris, pH 7.4. Specific probe (3×10^6 cpm/mL) was added, and the blots were allowed to hybridize for 24 hours at 50°C. The blots were washed three times for 5 minutes each in 2X SSC and 0.1% sodium dodecyl sulfate (SDS) at room temperature and three times for 30 minutes each in 0.1X SSC and 0.1% SDS at 60°C followed by autoradiography at −70°C. Sequential hybridizations to the probes were made by erasing the blots by two exposures of 15 minutes each in 0.1% SDS at 95°C. mRNA levels were quantified by densitometry using a Hewlett-Packard (Boise, ID) Scan Jet 4c scanner equipped with a transilluminator and image analysis software (SigmaGel; Jandel Scientific, San Rafael, CA). Each sample represented RNA from epididymal adipose tissue of a single rat.

Data Analysis

Data were compared by the two-tailed unpaired *t* test or one-way ANOVA. Curve-fitting was performed by power series analysis (nonlinear fitting) or linear regression.

RESULTS

Group I Experiments

STZ treatment administered on day −2 resulted in rapid weight loss (Table 1). Tail vein blood glucose increased to greater than 300 mg/100 mL in all rats treated with STZ. In rats killed at the time of diagnosis of diabetes (day 0), insulin concentrations in plasma obtained by cardiac puncture were reduced to the lower range of detection. Treatment of the diabetic rats with insulin resulted in hyperinsulinemia relative to nondiabetic control rats, rapid improvement in glycemia, and a gradual increase in body weight (Table 1 and Fig 1). Sham-STZ, sham-insulin–treated control rats also gained weight, as expected for ad libitum–fed Sprague-Dawley rats of the age studied. Glycemia at the time of death in insulin-treated diabetic rats (day 2 through 17) was lower (but not significantly) and more variable than in the control (day −2 and day 17) rats (mean ± SEM, 148 ± 39 *v* 166 ± 11 mg/100 mL).

Plasma leptin concentrations were markedly reduced in insulin-deficient diabetic rats compared with nondiabetic control rats (Fig 1). Insulin treatment of diabetic rats resulted in rapid restoration of plasma leptin by day 2 of treatment, followed by a further increase by day 4 to concentrations greater than twofold above both the prediabetic and the 17-day sham-treated controls. Hence, the restoration and subsequent

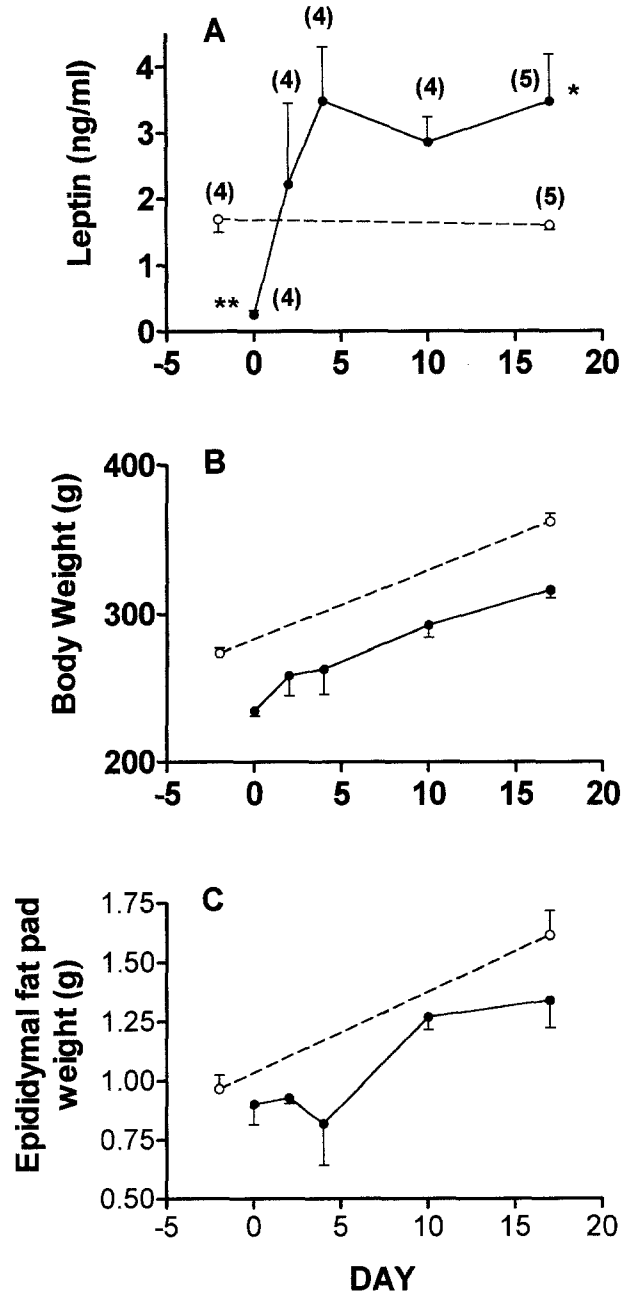

Fig 1. Plasma leptin in group I rats in comparison to body weight and epididymal adipose tissue mass plotted over time in (○- - -○) control (day −2 and 17), (●—●) STZ-diabetic (day 0) and insulin-treated STZ-diabetic rats (days 2 to 17). Data represent the mean × SEM (A) plasma leptin, (B) body weight, and (C) epididymal adipose tissue mass in groups of rats killed at the times indicated. *$P < .05$ *v* nondiabetic rats killed on day 17; **$P < .001$ *v* nondiabetic rats killed on day −2. Number in parentheses specifies n for each point.

further increase or overshoot in plasma leptin occurred prior to recovery of body weight to prediabetic levels.

By approximately 10 days of insulin treatment, diabetic rats had regained body weight lost as a result of the diabetes (Fig 1). Thereafter, they continued to gain weight, although they did not achieve the "catch-up" weight equivalent to the sham-treated controls. Nonetheless, the rate of weight gain in diabetic rats appeared constant and equivalent to the rate of weight gain in sham-controls. Hence, this parallel increase in body weight in insulin-treated diabetic rats occurred despite plasma leptin concentrations substantially above the sham-control level at each time point examined. Changes in adipose fat mass as reflected by epididymal fat pad weight occurred in proportion to weight gain (Fig 1).

In addition to the body weight measured at death, each animal was weighed at multiple time points. A similar rate of weight gain was observed in rats treated with insulin for 2, 4, 10, and 17 days that occurred in parallel to the sham-control group (data not shown).

Plasma leptin concentrations were examined in relation to plasma insulin and glucose determined in the same plasma sample in all rats studied (Fig 2). Plasma leptin showed a significant positive linear relationship to insulin ($r^2 = .45$, $P < .001$). Plasma leptin also showed a significant negative linear correlation with glucose ($r^2 = .61$, $P < .001$); however, this relationship was stronger ($r^2 = .68$) when expressed as a curvilinear function.

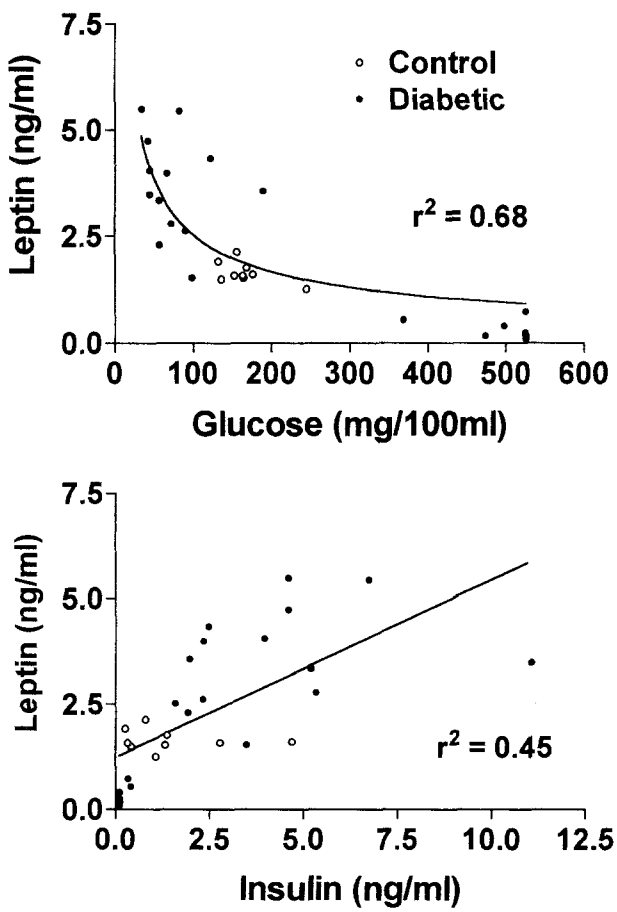

Fig 2. Plasma leptin in group I rats as a function of (A) plasma insulin and (B) plasma glucose. All 3 parameters were assayed in blood samples obtained by cardiac puncture immediately after methoxyflurane anesthesia. Data points include all rats studied (diabetic, insulin-treated diabetic, and controls).

Northern blot analysis was used to measure leptin mRNA content in epididymal adipose tissue of 17-day insulin-treated diabetic rats compared with 17-day sham-controls (Fig 3). No difference in leptin message was observed between these groups when normalized to either adipsin, GLUT-4, or total RNA loaded. We have previously shown that adipose GLUT-4 mRNA levels in STZ-diabetic rats subjected to prolonged insulin therapy return to baseline values after 7 days.[14] Hence, GLUT-4 mRNA represents a reasonable adipose cell–specific marker for normalization of leptin mRNA in the current study.

Group II Experiments

Since glucose concentrations were low (compared with controls) in most insulin-treated group I diabetic rats (Fig 2), the question arose as to whether hypoglycemia might be important for increasing plasma leptin. Therefore, we measured plasma leptin levels in STZ-diabetic rats infused with insulin while glucose was prevented from decreasing to less than 200 mg/100 mL (clamped at a target glycemia of 200) or allowed to decrease to 40 mg/100 mL (clamped at a target glycemia of 40 mg/100 mL). In rats targeted to either level of glycemia, plasma leptin increased within 1 hour and continued to increase steadily over the 3-hour infusion period (Fig 4). As expected, considerably more glucose was required to maintain glucose levels at 200 versus 40 mg/100 mL. Mean insulinemia was higher (but not significantly) in rats targeted to glycemia of 40 mg/100 mL versus rats targeted to 200 mg/100 mL even though the infusion rats were the same (Table 2). The reason for the variability in measured insulin concentrations is unclear, but all samples in insulin-infused rats had to be diluted 100-fold to obtain readings within the assay range, and insulinemia was markedly higher than basal for diabetic or nondiabetic rats in all insulin-infused animals.

To exclude effects of the animal procedures per se on leptin secretion, additional STZ-diabetic rats were subjected to a sham protocol including infusion of saline rather than insulin and glucose. Plasma leptin in sham-treated rats did not increase above baseline (Fig 4), whereas plasma leptin in insulin/glucose-treated rats increased rapidly, as expected. Hence, plasma leptin was not affected by the stress of the operative procedures.

To compare the leptin response to insulin between rats targeted to 200 mg/100 mL or 40 mg/100/mL glucose and the sham-infused group, we determined the mean slope of the leptin response to insulin for each group (Fig 4). These values in the insulin-treated groups (1.75 ± 0.22 ng/mL/min $\times 10^{-2}$ for rats targeted to 200 mg glucose/100 mL and 2.60 ± 0.38 for rats targeted to 40 mg/100 mL) did not differ significantly from each other, but were both increased markedly compared with values in the sham-treated rats (-0.14 ± 0.17, ANOVA, $P < .001$).

Finally, we examined the effect of insulin infusion with glycemia targeted to 200 mg/100 mL on plasma leptin in

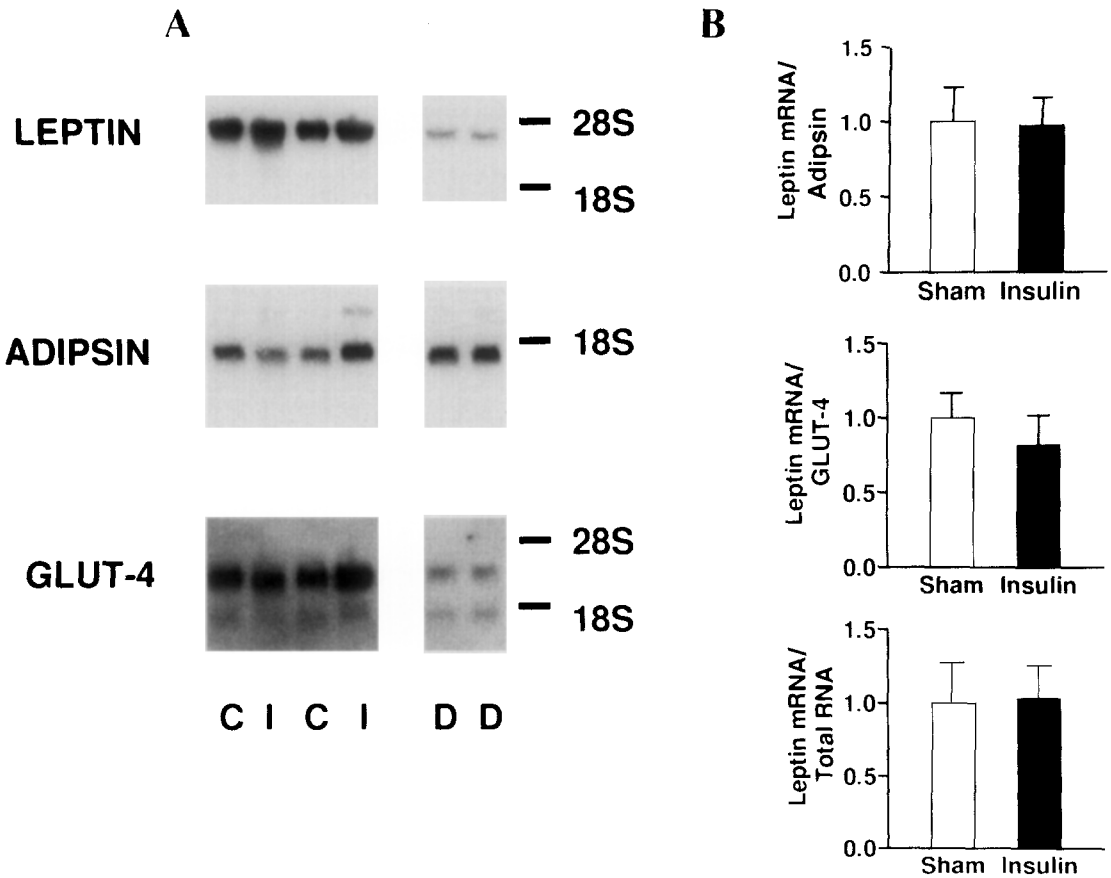

Fig 3. Leptin mRNA expression (relative density) by Northern blot analysis in 17-day insulin-treated, group I diabetic rats, and sham-treated controls. (A) Epididymal adipose tissue leptin, adipsin, and GLUT-4 mRNA levels in rats made diabetic with STZ and subsequently treated for 17 days with insulin (I) *v* sham-controls (C). Message expression in 2 untreated diabetic rats (D; tested on the same blot) is shown for comparison. Total epididymal adipose tissue RNA (15 μg) was hybridized to rat leptin cDNA, erased, and sequentially hybridized to cDNAs encoding rat adipsin and rat GLUT-4. (B) Leptin mRNA levels normalized to adipsin, GLUT-4, and total RNA loaded in 17-day insulin-treated diabetic rats *v* sham-controls (mean × SEM, n = 5 for each group). No significant differences in leptin mRNA were observed between insulin-treated diabetic and control rats.

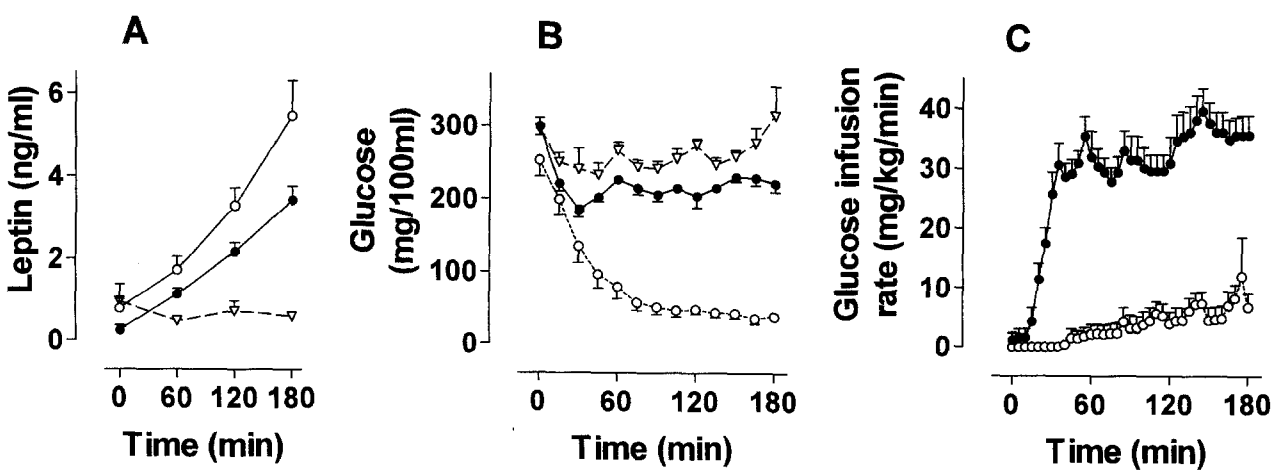

Fig 4. STZ-diabetic rats (Table 2) were treated with (--▽--) a 3-hour constant insulin infusion (1.0 U/h) and variable-rate glucose to target glycemia at (—●—) 200 (n = 7) or (--○--) 40 (n = 7) mg/100 mL. Sham-treated STZ-diabetic rats (n = 6) were exposed to the same operative procedure but received vehicle rather than insulin plus glucose. (A) Plasma leptin concentration; (B) glucose; (C) glucose infusion rate. Data represent the mean × SEM.

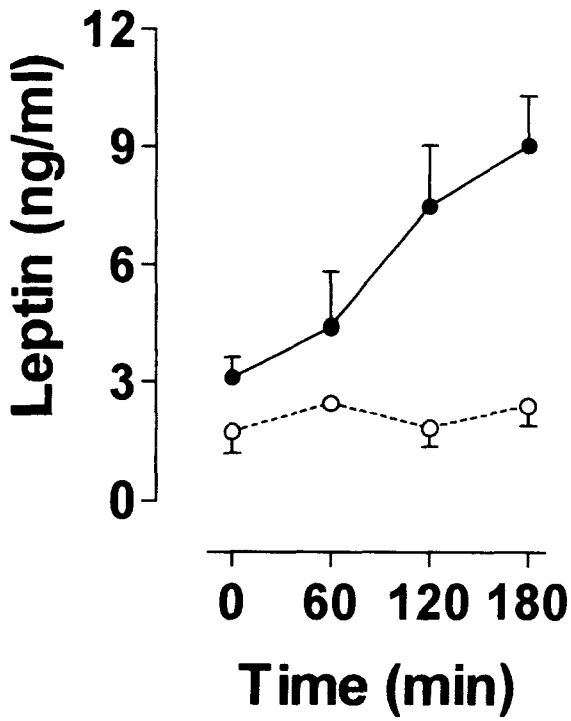

Fig 5. Plasma leptin concentrations in nondiabetic rats treated with insulin and glucose (n = 4; ●—●) compared with sham-treated nondiabetic controls (n = 5; ○---○). Rats were treated with a 3-hour constant insulin infusion (1.0 U/h) and variable-rate glucose with glycemia targeted at 200 mg/100 mL. Sham-treated rats were exposed to the same operative procedure but received vehicle rather than insulin plus glucose.

nondiabetic rats (mean weight, 411 ± 5 g; n = 4). As in the diabetic rats, plasma leptin rapidly increased (Fig 5). In these rats, initial plasma leptin (time 0) was higher than in the diabetic rats (consistent with the data in Fig 1) and increased to a higher concentration than in the diabetic rats after insulin/glucose infusion (compare Figs 4 and 5). In contrast, there was no change in plasma leptin in rats (mean weight, 411 ± 4 g; n = 5) subjected to sham-experimental procedures, again confirming that plasma leptin is unaffected by these procedures per se. The mean slope of the leptin response curves (Fig 5) was 3.53 ± 0.054 ng/mL/min × 10^{-2} for insulin/glucose-infused rats, compared with 0.06 ± 0.31 for sham-infused rats ($P < .001$ by unpaired t test). Insulinemia in insulin-infused normal rats (mean ± SE, 103 ± 48 ng/mL) was comparable to that in the insulin-infused diabetic rats and much higher than in sham-treated normal rats (1.16 ± 0.16 ng/mL).

DISCUSSION

STZ-induced diabetes with weight loss resulted in a markedly reduced plasma leptin concentration. This was reversed by 2 days of subcutaneous insulin therapy, and continued insulin treatment increased plasma leptin considerably above baseline (prediabetic) concentrations. This occurred before body weight or epididymal fat mass returned to baseline, suggesting that insulin itself increased circulating leptin independently of altered adipose mass.

The observed decrease in plasma leptin in STZ-diabetic rats is consistent with the decreased epididymal adipose tissue leptin mRNA levels previously reported by our laboratory[4] and others.[5,6] Becker et al[5] also reported a decrease in subcutaneous (inguinal) adipose leptin mRNA in STZ-diabetic rats. This decrease in leptin mRNA is not rapidly reversible by insulin therapy, and restoration to baseline (nondiabetic levels) does not occur in rats prior to restoration of body weight.[4,5] In the current study, we continued insulin therapy for 17 days to a point at which body weight and epididymal fat mass did recover, at least to baseline levels. Under these conditions, epididymal adipose mRNA also returned to baseline. However, unlike plasma leptin, an overshoot in leptin mRNA above baseline did not occur. Hence, these findings suggest that the increase in plasma leptin induced by insulin therapy does not depend on steady-state message levels, at least within the adipose tissue depots examined, and may depend on posttranscriptional cellular events regulating the translation, intracellular pooling, or peptide release or altered clearance of circulating leptin. More investigation will be needed to sort out these possibilities.

There is further support for the disparity between plasma leptin and leptin mRNA observed in the current study. The increase in plasma leptin in insulin-treated STZ-diabetic rats was evident as early as 2 to 4 days (Fig 1). However, our prior studies[4] showed that leptin mRNA remained well below prediabetic levels after 2 days of insulin treatment, and Becker et al[5] found that leptin mRNA in STZ-diabetic rats treated with insulin for up to 4 days still remained below the levels in nondiabetic rats. Hence, our current 2- to 4-day plasma leptin results considered along with past studies of leptin message support the concept of a discordance of circulating leptin and leptin message in insulin-treated diabetic rats. There is little additional information regarding concurrent changes in plasma leptin and leptin message in either rodents or humans. Clapham et al[16] measured plasma leptin and subcutaneous adipose tissue leptin mRNA in nondiabetic lean and obese humans before and after a mixed meal and observed no changes in either parameter despite a substantial increase in plasma insulin. However, there may be species differences in the rate of leptin response to insulin (described later).

Although insulin-treated STZ-diabetic rats never achieved a body weight equal to that of the sham-treated controls, they did gain weight and epididymal adipose mass at the same rate. This occurred despite elevated plasma leptin from days 4 to 17. Hence, the parallel increase in weight and epididymal fat mass in the presence of hyperleptinemia suggests that moderately elevated leptin concentrations do not affect food intake or energy disposal. Alternatively, other factors may offset these actions of leptin, resulting in a resistance to leptin action perhaps similar to what has been postulated to occur in human and rodent obesity.[17-19]

Given that leptin appears to be a hypothalamic signal to reduce food intake, plasma leptin might be expected to be low under conditions of hypoglycemia. However, the hyperleptinemia in our group I rats was in most cases associated with

glucose levels less than the sham-treated control levels (Fig 2), suggesting that relative hypoglycemia might actually have increased plasma leptin. However, based on our group II studies, this does not appear to be the case. In these experiments, insulin infusion rapidly increased plasma leptin in STZ-diabetic rats irrespective of whether glycemia was prevented from declining to less than 200 mg/100 mL or allowed to decrease to 40 mg/100 mL. These data show that hypoglycemia was not necessary to increase leptin and suggest that insulin, not glucose, appears to be the important factor. Nonetheless, considering the inhibitory action of leptin on food intake, it is of interest that intravenous insulin did in fact increase plasma leptin and that hyperleptinemia (relative to nondiabetic controls) was in fact evident in subcutaneous insulin-treated diabetic rats despite hypoglycemia. We acknowledge that the clamp conditions used in the group II studies do not precisely reproduce the subcutaneous insulin treatment administered to the group I rats, so it remains possible that group I rats may have had repeated or more prolonged hypoglycemia, which conceivably could have had different effects on plasma leptin.

Taken together, our group I and II studies suggest that plasma leptin is volatile in diabetic rats, varying between low and high concentrations (relative to nondiabetic rats) depending on exogenous insulin replacement. It is possible that plasma leptin is also volatile in human type I diabetes; however, this will require direct testing in human subjects.

Tuominen et al[20] reported that fasting plasma leptin was elevated in human subjects with type I diabetes compared with nondiabetic controls of similar body weight and fat content. Four hours of hyperinsulinemia at clamped euglycemia did not increase leptin in diabetic subjects and resulted in only a modest (25%) increase in control subjects. Dagogo-Jack et al[21] reported that plasma leptin does not change in humans with or without type I diabetes as a result of hyperinsulinemia at normoglycemia or hypoglycemia. Other investigators[16,22,23] also noted no acute effect of insulin to increase plasma leptin in humans. However, prolonged and high-dose insulin administration to humans did appear to increase plasma leptin.[22,24,25] Our finding of increased leptin in insulin-treated STZ-diabetes is consistent with the findings of Tuominen et al in type I human diabetes. However, our rodent results are different from the human studies in terms of responsiveness to intravenous insulin. Whereas the human studies imply that in vivo leptin responsiveness to insulin requires prolonged insulin administration, we observed a rapid and substantial increase in leptin in both STZ-diabetic and nondiabetic rats (Figs 4 and 5). Thus, there may be species differences in insulin-mediated leptin release. There is little additional information concerning the effect of insulin on plasma leptin in diabetic or normal rodents. Hardie et al[26] reported that insulin increased plasma leptin in lean fa/fa rats. Only a time point at 4 hours post–insulin injection was examined in that study.

Certain factors could explain the discrepancy in insulin responsiveness between rodents and humans. It is known that insulin-induced glucose flux, measured as whole-body glucose utilization, is higher in rodents than in humans even at equivalent glycemia.[27] Hence, there may be a critical degree of insulin-induced energy uptake beyond which leptin release is triggered. In our rodent studies, insulin concentrations were roughly 10- to 100-fold above those achieved in the studies by Tuominen et al,[20] but were within the range of several insulin concentrations studied by Kolaczynski et al[22] in short-term clamp studies wherein leptin did not increase.

Adipose stores in our diabetic rats subjected to intravenous insulin infusion studies were probably depleted relative to control levels. Although not directly measured, this seems likely, since body weight decreased markedly after STZ administration and was not restored to baseline levels at the time of study. This suggests that the absolute level of adipose cell energy storage may not be as critical for fat cell leptin release as the level of circulating insulin or the rate of insulin-induced energy entry or energy flux. We cannot exclude potential effects of other insulin- or glucose-responsive factors in modulating the leptin response to insulin in this in vivo study. In particular, serum cortisol, which is known to stimulate adipose leptin production,[28] may have played a role. This would more likely have occurred at the glycemia target of 40 mg/100 mL, but would less likely have contributed to the potent leptin response also observed at 200 mg/100 mL.

It has been proposed that leptin might be considered a glucose counterregulatory hormone.[29] The increase in leptin with insulin treatment associated with low glucose observed in our group I experiments is consistent with this concept. This would also be consistent with recent findings by Cohen et al[30] suggesting that leptin may inhibit several cellular steps mediating insulin signal transduction and with findings by Emilsson et al[31] indicating that leptin impairs insulin secretion in the perfused pancreas prepared from *Lep^{ob}/Lep^{ob}* mice. On the other hand, a glucose counterregulatory role for leptin is not supported by our observations that leptin also increased considerably when glycemia was prevented from decreasing to less than 200 mg/100 mL. Further, recent studies of adenoviral leptin–transfected rats,[32] as well as studies in our laboratory of rats treated with subcutaneous leptin or exposed to hyperinsulinemic glucose clamping,[33] show that leptin enhances rather than impairs insulin sensitivity. Hence, the physiologic role of leptin secreted in response to insulin still needs clarification.

In summary, our results provide new information concerning the in vivo physiologic control of circulating leptin concentrations. First, plasma leptin concentrations are volatile in insulin-treated diabetic rats, being markedly reduced under conditions of insulin deficiency and rapidly increased to levels above control with insulin treatment. Plasma leptin is also susceptible to rapid change in normal rats, increasing rapidly with insulin infusion. This volatility in responsiveness to insulin differs from what has been reported in human studies, wherein insulin's effects on plasma leptin appear more gradual. Second, the increase in plasma leptin in insulin-treated diabetic rats does not require restoration of body weight, may occur even in the presence of hypoglycemia, and, under clamped glycemia, does not depend on target glucose. Finally, hyperleptinemia in insulin-treated diabetic rats is not explained on the basis of steady-state leptin mRNA, at least as reflected in epididymal fat.

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