Rapid Publication

Leptin Restores Euglycemia and Normalizes Glucose Turnover in Insulin-Deficient Diabetes in the Rat

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Leptin has been shown to improve insulin sensitivity and glucose metabolism in normoinsulinemic healthy or obese rodents. It has not been determined whether leptin may act independently of insulin in regulating energy metabolism in vivo. The present study was designed to examine the effects of leptin treatment alone on glucose metabolism in insulin-deficient streptozotocin (STZ)-induced diabetic rats. Four groups of STZ-induced diabetic rats were studied: *1*) rats treated with recombinant methionine murine leptin subcutaneous infusion with osmotic pumps for 12–14 days (LEP; 4 mg · kg^{-1} · day^{-1}, *n* = 10); *2*) control rats infused with vehicle (phosphate-buffered saline) for 12–14 days (VEH; *n* = 10); *3*) pair-fed control rats given a daily food ration matching that of LEP rats for 12–14 days (PF; *n* = 8); and *4*) rats treated with subcutaneous phloridzin for 4 days (PLZ; 0.4 g/kg twice daily, *n* = 10). Phloridzin treatment normalizes blood glucose without insulin and was used as a control for the effect of leptin in correcting hyperglycemia. All animals were then studied with a hyperinsulinemic-euglycemic clamp (6 mU · kg^{-1} · min^{-1}). Our study demonstrates that leptin treatment in the insulin-deficient diabetic rats restored euglycemia, minimized body weight loss due to food restriction, substantially improved glucose metabolic rates during the postabsorptive state, and restored insulin sensitivities at the levels of the liver and the peripheral tissues during the glucose clamp. The effects on glucose turnover are largely independent of food restriction and changes in blood glucose concentration, as evidenced by the minimal improvement of insulin action and glucose turnover parameters in the PF and PLZ groups. Our results suggest that the antidiabetic effects of leptin are achieved through both an insulin-independent and an insulin-sensitizing mechanism. *Diabetes* 48:XXX–XXX, 1999

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LEP, rats treated with leptin; PF, pair-fed control rats; PLZ, rats treated with phloridzin; R_a, rate of glucose appearance; R_d, rate of glucose disappearance; STZ, streptozotocin; T4, thyroid hormone; VEH, control rats infused with vehicle.

The effects and mechanisms of leptin in modulating insulin actions and regulating glucose metabolism are still being debated. Even though several in vitro studies suggested an insulin-antagonizing action of leptin in a number of tissue preparations (1,2), most in vivo studies point to an insulin-enhancing action of leptin at both the whole body (3–7) and the tissue (8–10) levels. The in vivo effects of leptin on energy metabolism have been achieved either by peripheral (subcutaneous and intravenous) administration at sufficient doses (3–5) (11) or by central administration (injection into a lateral cerebral ventricle) at doses that are hundreds of times less than those used in the peripheral administration (6,9,11). Leptin-treated animals required substantially increased amounts of glucose infusion during hyperinsulinemic-euglycemic clamps, which were found to be associated with enhanced insulin sensitivity at the levels of both the liver and the peripheral tissues, especially the skeletal muscle (3,5,6). Enhanced hepatic insulin sensitivity results in an augmented insulin-mediated suppression of hepatic glucose production, whereas enhanced insulin sensitivity in peripheral tissues is associated with a greater extraction of glucose from the circulation. Recently, we also found that the effects of leptin are differential, dependent upon the specific tissue types, in modulating the actions of insulin in glucose and energy metabolism (10). Our results demonstrate that the differential action mechanism of leptin enhances insulin's stimulation of glucose utilization in the brown adipose tissue and skeletal muscle, but antagonizes insulin's promotion of energy storage in the white fat depot.

In in vivo studies, leptin has been given mostly to normal or obese animals that have normal, or even elevated, insulin secretion. Although leptin was shown to suppress insulin secretion, and the effects of leptin could be obtained in the face of reduced circulating insulin levels, it has not been determined whether the actions of leptin can be achieved independently of insulin. In the present study, we wanted to determine whether leptin could act independently of insulin to modulate glucose metabolism in an insulin-deficient diabetic model in the rat. This study was conducted in streptozotocin (STZ)-induced diabetic rats featuring moderate insulin deficiency and significant hyperglycemia (12) and many pathological and biochemical changes that resemble human type 1 diabetes (13). The effects of leptin on food intake, body weight change, blood glucose, and insulin levels were monitored and compared with the vehicle and pair-fed

control animals. At the end of the treatment, the impact of leptin on insulin action and glucose turnover was evaluated with hyperinsulinemic-euglycemic clamps. Since leptin treatment in our preliminary study normalized fasting hyperglycemia in this diabetic model (14), it is important for us to determine whether the effect of leptin on whole body glucose metabolism may be partly dependent on the restoration of euglycemia. Thus, we included another group of STZ-induced diabetic rats with phloridzin treatment to serve as a euglycemic control. Phloridzin normalizes blood glucose by increasing glycosuria, and has been accepted as an effective means of restoring euglycemia in diabetic rats without the use of insulin (12,15).

RESEARCH DESIGN AND METHODS

Animal preparation. Male young adult Sprague Dawley rats (body weight 373 ± 7 g) were obtained from Harlan Sprague Dawley (San Diego, CA) and were maintained in light- and temperature-controlled facilities. All animals were fed a balanced regular diet, with drinking water given ad libitum. Insulin-deficient diabetes was induced with a single bolus intraperitoneal injection of STZ (50 mg/kg; Sigma, St. Louis, MO), which resulted in marked hyperglycemia within 2 days and persisted without treatment throughout the duration of the study. After establishing basal hyperglycemia, the animals were randomly divided into four groups, including *1*) rats treated for 12–14 days with subcutaneous recombinant murine leptin (r-metMuLeptin; Amgen, Thousand Oaks, CA) infusion at 4 mg $\cdot$ kg^{-1} $\cdot$ day^{-1} using subcutaneous Alzet osmotic pumps (LEP, n = 10); *2*) control rats infused with vehicle (phosphate-buffered saline) for 12–14 days (VEH, n = 10); *3*) pair-fed (with vehicle) control rats receiving for 12–14 days a daily food ration that matched that of the LEP group (PF, n = 8); and *4*) diabetic rats treated with phloridzin for 4 days (PLZ, n = 10). Phloridzin was dissolved in propylene glycol (40% wt/vol) and given subcutaneously at a dose of 0.4 g/kg twice daily for 4 days. Body weight, food intake, and blood glucose levels (Glucometer Elite; Bayer, Elkhart, IN) were monitored daily.

All animals were brought to a general anesthesia ~5–7 days before the glucose clamp experiments, and received chronic cannulation of the left carotid artery and right jugular vein using PE-50 intramedic tubing fitted with a short segment of medical silicone tubing (0.020"/0.037" ID/OD; Baxter, Hayward, CA) for a flexible noninjurious insertion into the vessels. The cannulas were tunneled subcutaneously and exteriorized from the back of the neck. The cannulas were filled with a mixed saline solution containing 60% (wt/vol) polyvinylpyrrolidone (Sigma) and 100 U/ml heparin.

Hyperinsulinemic-euglycemic clamp experiments. Experiments were conducted in the conscious and freely moving animals after they were fasted for 5–6 h. Contents in the vascular cannulas were aspirated, and the cannulas were extended with PE-50 plastic tubing and flushed with heparinized (10 U/ml) saline. The right jugular vein cannula was used for infusion of tracer, insulin, and glucose via two serial T-shaped needle connectors. The experiments started with a primed constant infusion (3.6 µCi/bolus + 0.07 µCi/min) of 3-[^{3}H]glucose (NEN, Boston, MA) for determination of systemic glucose appearance and disappearance. The experiment consisted of a tracer equilibration period (from –120 to –60 min), a basal sampling period (–60 to 0 min), and a hyperinsulinemic-euglycemic clamp period (0–180 min). A constant porcine insulin infusion (Lilly, Indianapolis, IN) was begun at t = 0 min and maintained at 6 mU $\cdot$ kg^{-1} $\cdot$ min^{-1} until the end of the experiment. An exogenous glucose infusion (30%) was given at variable rates according to instant plasma glucose measurements to achieve and/or maintain normal plasma glucose levels (100–115 mg/dl). Arterial blood from the carotid cannula was sampled every 10 min during the basal and glucose clamp periods. Fresh blood was centrifuged using a microfuge, and plasma glucose was immediately measured. Plasma was also collected in ice-chilled Eppendorf microtubes containing sodium fluoride (for glucose tracer measurements) or EDTA/approtinin (for hormones and metabolites), and stored at –70°C until assayed. The packed blood cells, after removal of plasma from the blood samples, were resuspended in heparinized saline and reinfused after each blood sampling to prevent volume depletion and anemia (6,12).

Laboratory methods. Plasma glucose concentrations were measured by a glucose oxidase method using the Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA). Plasma glucose specific activity was derived from plasma glucose concentration and 3-[^{3}H]glucose radioactivity determined by a liquid scintillation method on a Beckman LS60001C Counter as described (6). The plasma samples were deproteinized with $Ba(OH)_2$ and $ZnSO_4$, and the supernatant was evaporated overnight at 70°C. The rates of glucose appearance (R_a) and disappearance (R_d) were calculated with an equation for non–steady-state turnover using a one-compartment model (16) that has been validated (17). Raw data for glucose concentration and specific activity were smoothed according to the optimal segments method (18). During the basal period, R_a was equated to hepatic glucose production. During the hyperinsulinemic-euglycemic clamp, glucose production was calculated as the difference between R_a and rate of exogenous glucose infusion. Tracer-derived R_d in the hyperglycemic rats during the basal period was the sum of systemic glucose utilization and glycosuria. Our experimental procedure did not allow separate quantitation of glycosuria and glucose utilization. During the euglycemic clamp, R_d was equated to whole body glucose utilization.

Analyses of the biochemical parameters triglyceride, cholesterol, corticosterone, β-OH-butyrate, free fatty acids, lactate, and glycerol were performed spectrophotometrically on a Hitachi 717 Clinical Chemistry Autoanalyzer (Boehringer Mannheim, Indianapolis, IN). Plasma insulin concentration was measured with a Sensitive Rat Insulin Radioimmunoassay kit from Linco Research (St. Charles, MO), and plasma leptin concentration was determined by an enzyme-linked immunoassay (6,19).

All data are expressed as means ± SE. Two-way analysis of variance was used to determine statistical difference between the experimental groups. Significance is assumed at $P < 0.05$.

RESULTS

Insulin-deficient diabetes was associated with prominent hyperphagia, evidenced by a daily food consumption of 41.0 ± 0.9 g/day in the control (VEH) rats (Fig. 1). Leptin treatment resulted in a 50% reduction in daily food consumption (19.6 ± 0.4 g/day). PF animals were rationed daily with the same food amount as the LEP rats. PLZ animals consumed less food (33.8 ± 1.1 g/day) than the VEH control rats ($P < 0.05$), but more than LEP and PF rats ($P < 0.01$). Diabetes without treatment was associated with a body weight loss (VEH: –2.6 g/day; Fig. 1). Food restriction with pair-feeding appeared to worsen the body weight loss in the diabetic rat (–10.3 g/day, $P < 0.01$ vs. all other groups). In spite of the fact that LEP-treated rats consumed the same amount of food as the PF group, the body weight loss with leptin was only 40% that of the PF group ($P < 0.01$). Phloridzin treatments did not significantly accentuate the daily body weight loss seen with the VEH control rats.

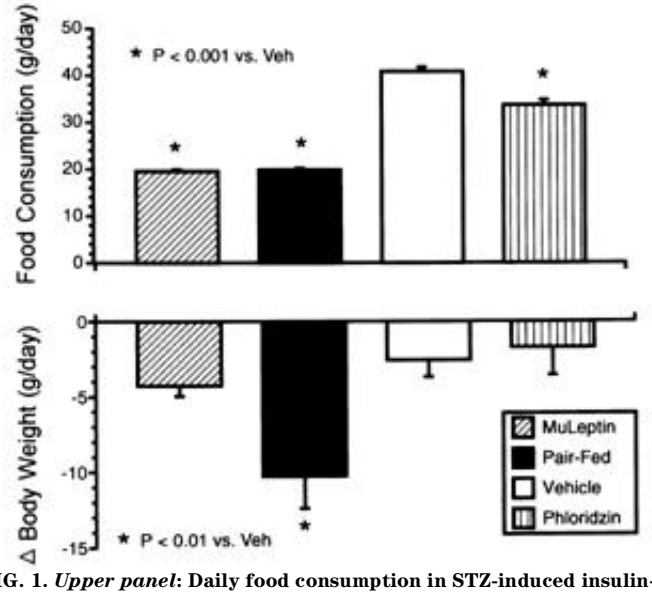

FIG. 1. *Upper panel*: Daily food consumption in STZ-induced insulin-deficient diabetic rats treated with subcutaneous infusions of murine leptin (MuLeptin; n = 10), phosphated buffer (Vehicle; n = 10), vehicle plus pair-feeding (n = 8), or subcutaneous phloridzin (n = 9). *Lower panel*: Changes in daily body weight with each of the treatment modalities. All values are expressed in means ± SE. *Statistical significance at $P < 0.01$ for comparisons between VEH and each of the other three groups.

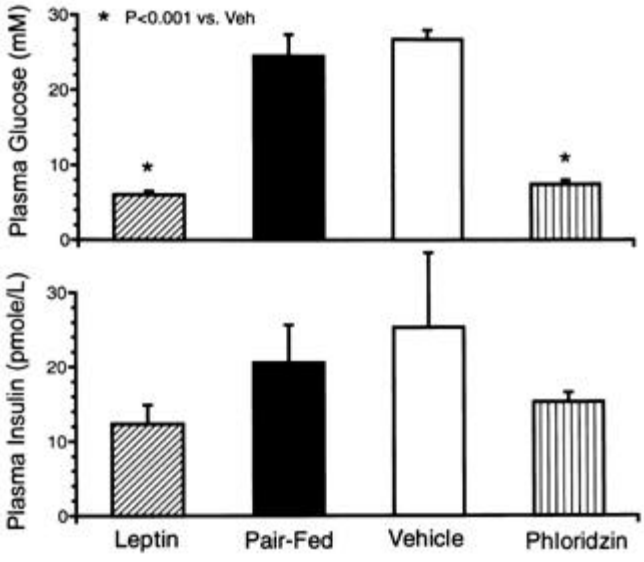

FIG. 2. ***Upper panel*****: Fasting plasma glucose levels on the day of the experiment after respective treatment of subcutaneous infusions of murine leptin (*n* = 10), phosphated buffer (Vehicle, *n* = 10), vehicle plus pair-feeding (*n* = 8), or subcutaneous phloridzin (*n* = 9) in STZ-induced insulin-deficient diabetic rats. ****P* < 0.001 vs. VEH.** ***Lower panel*****: Changes in daily body weight with each of the treatment modalities. All values are expressed in means ± SE.**

On the day of the experiment, fasting hyperglycemia was prominent in the untreated diabetic rats (VEH: 26.9 ± 1.3 mM; Fig. 2). Food restriction in the PF animals had little, if any, impact on the high glucose levels (24.6 ± 2.9 mmol/l, NS vs. VEH). Chronic infusion of leptin restored fasting normoglycemia at 6.1 ± 0.4 mmol/l (P < 0.0001 vs. VEH and PF). Phloridzin treatment also rendered the diabetic animals euglycemic (7.3 ± .07 mmol/l, P < 0.001 vs. VEH and PF). STZ-induced diabetes is characterized by significantly attenuated fasting plasma insulin levels, as seen in the VEH group (25.4 ± 10.0 pmol/l; Fig. 2), which were only 5–10% of the normal values reported in our recent study (6). Fasting plasma insulin levels in diabetic rats were moderately but not significantly reduced by leptin (12.4 ± 2.5 pmol/l), pair-feeding (20.6 ± 5.1 pmol/l), and phloridzin (15.3 ± 1.3 pmol/l).

The untreated diabetic VEH rats displayed a severe insulin resistance, as evidenced by a minimal requirement of glucose infusion (8.4 ± 3.1 μmol · kg^{-1} · min^{-1}) during the hyperinsulinemic-euglycemic clamp (Fig. 3). Both pair-feeding and phloridzin treatments resulted in moderate and comparable increments in glucose infusion rates (76.5 ± 6.4 and 76.4 ± 4.0 μmol · kg^{-1} · min^{-1}, respectively, at t = 150–180 min). Leptin treatment was associated with a tremendous increase in the rate of glucose infusion (182.9 ± 3.1 μmol · kg^{-1} · min^{-1}), which was >20 times greater than vehicle and 2.4 times greater than phloridzin and pair-feeding.

In the postabsorptive state, the basal glucose production rate (R_a), an important driving force for fasting hyperglycemia, was elevated as expected in the untreated diabetic VEH rats (126.7 ± 8.3 μmol · kg^{-1} · min^{-1}; Fig. 4), which is more than twice the values reported in the normal rats in our recent study (6). The abnormally high rate of glucose production in diabetes was necessary to compensate for the urinary loss of glucose due to hyperglycemia. The elevated R_a was not affected by food restriction with PF (118.4 ± 6.5 μmol · kg^{-1} · min^{-1}, NS vs. VEH) rats. Correction of hyperglycemia with phloridzin was able to moderately improve the elevated basal glucose production (73.9 ± 3.9 μmol · kg^{-1} · min^{-1}, P < 0.001 vs. VEH and PF). Leptin treatment substantially improved the basal hepatic glucose production (54.4 ± 1.4 μmol · kg^{-1} · min^{-1}) to a level similar to that of normal rats as reported by us (6) and others (4). During the glucose clamp,

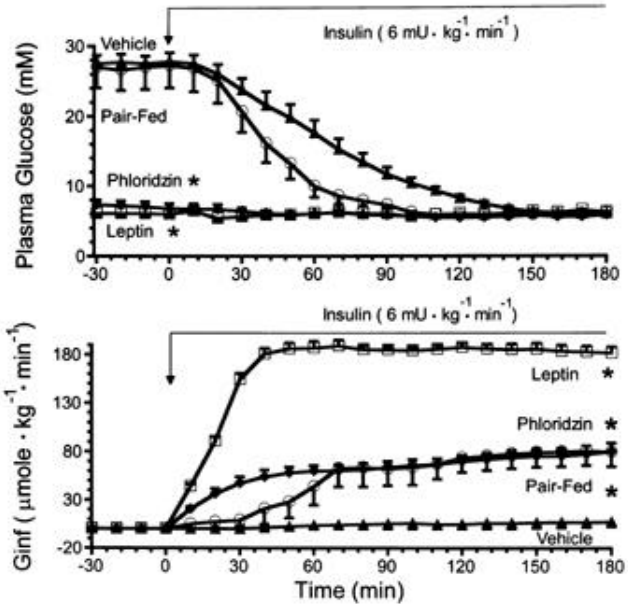

FIG. 3. ***Upper panel*****: Plasma glucose levels in the basal period and during the hyperinsulinemic-euglycemic clamp (6 mU · kg^{-1} · min^{-1}) in the STZ-induced diabetic rats with various treatments.** ***Lower panel*****: Glucose infusion rates (Ginf) during the hyperinsulinemic-euglycemic clamp. All values are expressed as means ± SE. *Statistical significance at P < 0.001 for comparisons between VEH and each of the other three groups, in the basal period (–30 to 0 min) for the upper panel and at the end of the glucose clamp (150–180 min) for the lower panel.**

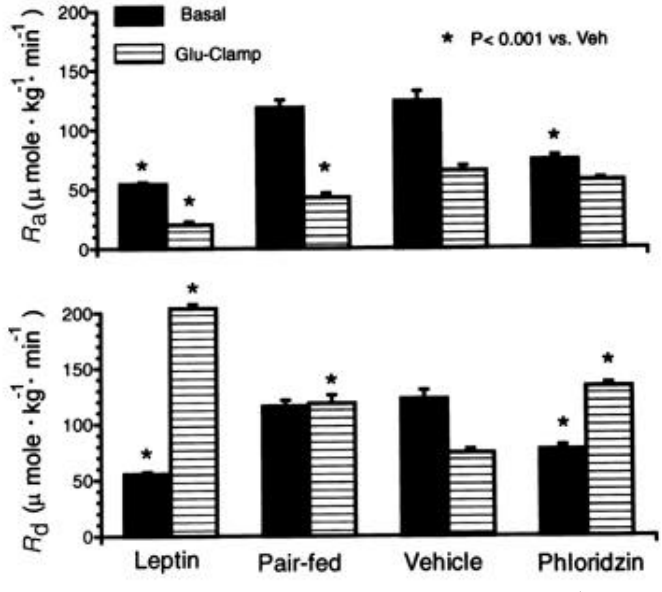

FIG. 4. Tracer-determined rates of glucose appearance (*R_a*; *upper panel*) and disappearance (*R_d*; *lower panel*) during the postabsorptive basal period (–30 to 0 min) and hyperinsulinemic-euglycemic clamp (6 mU · kg^{-1} · min^{-1}) period (150–180 min) in the STZ-induced diabetic rats with various treatments.

PF was associated with moderately improved hepatic insulin sensitivity, as evidenced by somewhat reduced hepatic glucose production in the PF group compared with that of untreated VEH rats (42.8 ± 3.5 vs. 64.9 ± 4.3 μmol · kg^{-1} · min^{-1}, $P < 0.001$). Correction of basal hyperglycemia with phloridzin did not affect hepatic glucose production (56.9 ± 2.0 μmol · kg^{-1} · min^{-1}). Leptin very markedly suppressed hepatic glucose production (20.1 ± 2.6 μmol · kg^{-1} · min^{-1}, $P < 0.01$ vs. all other groups), which was only 30% that of vehicle. Diabetic animals excrete large amounts of glucose in the urine. Quantitation of the urinary loss of glucose for a given time period in the nonanesthetized freely moving rats is very difficult, and was not performed in the present study. Therefore, the R_d values during the basal period were the sum of whole body glucose utilization and urinary glucose loss for the hyperglycemic groups (VEH and PF; Fig. 4). R_d rates objectively reflect systemic glucose utilization for LEP and PLZ groups during the basal period, and for all groups during the euglycemic clamp period, when glycosuria was obviated. It is apparent from Fig. 4 that under the high rate of insulin infusion, VEH rats displayed the lowest response to insulin, and hence the lowest glucose utilization rate (73.9 ± 3.5 μmol · kg^{-1} · min^{-1}). Both food restriction (PF: 118.4 ± 7.2 μmol · kg^{-1} · min^{-1}) and correction of hyperglycemia (PLZ: 133.2 ± 3.6 μmol · kg^{-1} · min^{-1}) resulted in moderate but significant increases in glucose utilization compared with VEH rats (both $P < 0.001$). Leptin treatment resulted in the greatest rate of glucose utilization (204.1 ± 3.6 μmol · kg^{-1} · min^{-1}), which was 1.7- and 1.5-fold greater than pair-feeding and phloridzin ($P < 0.05$), respectively, and was nearly threefold greater than vehicle ($P < 0.0001$).

With LEP, basal plasma levels of free fatty acids (0.3 ± 0.1 vs. 1.3 ± 0.3 mEq/l), glycerol (6.7 ± 0.8 vs. 33.7 ± 8.0 mg/dl), β-hydroxybutyrate (0.7 ± 0.1 vs. 28.6 ± 10.6 mg/dl), cholesterol (59.0 ± 4.3 vs. 75.2 ± 3.9 mg/dl), and triglyceride (7.9 ± 1.1 vs. 61.7 ± 14.5 mg/dl) were very markedly decreased from those seen in vehicle control animals ($P < 0.05$ or less), and were substantially lower than those in PF and PLZ groups ($P < 0.05$ or less for most parameters; Table 1). The elevated lactate levels due to food restriction seen in PF rats were not observed with LEP or PLZ. Plasma leptin levels were decreased in all STZ-induced diabetic rats (0.2–0.3 ng/ml) except the leptin-treated animals who displayed hyperleptinemia (65.3 ± 16.0 ng/ml; Table 1). Leptin treatment also caused a significant elevation in thyroid hormone (T4) levels compared with all other groups (Table 1).

DISCUSSION

The present study demonstrates for the first time that leptin treatment alone in the insulin-deficient STZ-induced diabetic rat results in a complete normalization of blood glucose and significant improvement in insulin sensitivity and glucose metabolism. STZ-induced diabetes has been a widely used animal model and is characterized by insulin deficiency, insulin resistance, and many other biochemical and pathological features shared by human type 1 diabetes (13). The insulin-deficient state is confirmed here by extremely reduced plasma insulin levels measured with a sensitive radioimmunoassay method. This study shows a greatly attenuated plasma leptin level in STZ-induced diabetic rats, in agreement with several recent reports (20,21). A decrease in leptin secretion may be related to a reduced adipose mass and reduced assimilation and storage of energy substrates in the fat tissue in insulin deficiency (20,21). Reduced leptin secretion, increased neuropeptide Y expression, and reduced corticotropin-releasing hormone expression may act in concert to cause hyperphagia in this STZ model of diabetes (22). Interestingly, significant reductions in circulating leptin levels have recently been documented in the newly diagnosed untreated human type 1 diabetic patients (23), suggesting that the reduced leptin secretion might be a common feature in insulin-deficient diabetes.

The results obtained from the present study using this insulin-deficient model of diabetes demonstrate a powerful action of leptin in the regulation of glucose metabolism. In severely insulinopenic rats, leptin treatment alone was able to correct hyperglycemia and restore basal euglycemia within 12–14 days, without the use of insulin or any other antidiabetic drugs. This suggests an insulin-independent mechanism of leptin action. During hyperinsulinemic-euglycemic clamp, leptin profoundly enhanced the effects of insulin in suppressing hepatic glucose production and in promoting whole body glucose disposal. This appears to argue for an insulin-sensitizing action mechanism for leptin. The effects of leptin are largely unaccounted for by food restriction, since pair-feeding resulted in only a small improvement in glucose turnover and had little, if any, effect on fasting hyperglycemia. In addition, leptin treatment minimized the body weight loss due to restriction of food intake seen with the pair-fed animals under insulin deficiency.

The most intriguing finding of this study is that leptin treatment alone results in a substantial improvement in glucose turnover (production and utilization) during the postabsorptive basal and hyperinsulinemic-euglycemic clamp periods. In the STZ-induced diabetic rats, severe peripheral insulin resistance is evidenced by a minimal glucose utilization rate, and hepatic insulin resistance is evidenced by a more than twice–elevated glucose production rate. After treating the diabetic animals with recombinant murine leptin for

TABLE 1
Plasma metabolites and hormones in STZ-induced diabetic rats

Treatment	Cholesterol (mg/dl)	Triglyceride (mg/dl)	Free fatty acids (mEq/l)	β-OH-Butyrate (mg/dl)	Lactate (mg/dl)	Glycerol (mg/dl)	Corticosterone (μg/dl)	Leptin (ng/ml)	T4 (μg/dl)
LEP	59.0 ± 4.3*†	7.9 ± 1.1*†	0.3 ± 0.1*‡	0.7 ± 0.1*	2.9 ± 0.3‡	6.7 ± 0.8*	16.1 ± 1.8	65.3 ± 16.0*†‡	2.6 ± 0.3*†‡
VEH	75.2 ± 3.9	61.7 ±14.5	1.3 ± 0.3	28.6 ± 10.6	4.0 ± 0.4	33.7 ± 8.0	16.1 ± 2.8	0.2 ± 0.1	1.2 ± 0.2
PF	67.9 ± 4.2	23.3 ± 2.1	1.2 ± 0.3	2.4 ± 0.6	5.5 ± 0.9	14.7 ± 1.3	13.9 ± 0.8	0.3 ± 0.2	1.7 ± 0.2
PLZ	86.6 ± 1.9	37.3 ± 3.1	1.0 ± 0.4	15.2 ± 2.6	1.7 ± 0.3	15.9 ± 1.1	13.4 ± 2.0	0.2 ± 0.02	0.9 ± 0.2

Data are means ± SE. *$P \leq 0.05$ vs. VEH; †$P \leq 0.05$ vs. PF; ‡$P \leq 0.05$ vs. PLZ.

12–14 days, the basal glucose production and utilization rates appeared to be normalized compared with the normal rats in our previous study (6). This is convincing evidence that leptin corrects hyperglycemia by restoring normal rates of glucose turnover. In normal rats, the effects of leptin in suppressing hepatic glucose production are believed to be due to marked inhibition of glycogenolysis (3), while relative contribution of gluconeogenesis to hepatic glucose production is increased (3,4). The improvement in peripheral tissue insulin sensitivity is reflected by a nearly threefold increase in whole body glucose utilization during insulin infusion, compared with the vehicle-treated diabetic control animals during insulin infusion. As demonstrated in earlier studies by us (10) and others (7,8), leptin enhances glucose uptake and oxygen consumption in a number of tissues, including skeletal muscle and brown adipose tissue, which may contribute to the increase in whole body glucose utilization seen in the current study and in many others (3,5,7).

Untreated insulin-deficient diabetes as seen in the vehicle group is characterized by elevated blood levels of triglyceride, free fatty acids, glycerol, and β-OH-butyrate due to insulin deficiency. Leptin treatment resulted in significant decreases in plasma triglyceride. This is consistent with a decreased triglyceride production rate, as seen in normal rats, consistent with our previous finding (24), and may also be related to an accelerated decomposition by increased lipoprotein lipase, which can be induced by leptin (25). In the normal rats, leptin causes gradual depletion of visible fat, especially in the visceral adipose tissues (3,11). This is associated with an escalation in lipolysis in the short term (3). In the long run, as the body fat mass diminishes, its lipolytic capacity might become increasingly limited. This may explain an increase in plasma free fatty acids with short-term leptin treatment, but in subjects with prolonged leptin treatment, free fatty acid levels may be found within the normal range (3,6), or even subnormal, as seen with the longer treatment in the present study. Similarly, the reduced glycerol and β-OH-butyrate can be consequences of a dwindled lipolysis and, hence, reduced free fatty acid oxidation under long-term leptin treatment. Leptin has recently been shown to inhibit fatty acid synthase in the adipocytes (25), which again might contribute to an overall reduction in fat mass and a decrease of free fatty acid release into circulation when a high rate of lipolysis can no longer be sustained. Increased levels of lactate, a product of elevated glycolysis, is believed to be an impaired glucose oxidative metabolism in muscles in diabetes (26) and has been demonstrated in STZ-induced diabetic rats (27). This is in agreement with an augmented Cori cycle, which contributes to an increased hepatic glucose production.

Interestingly, plasma T4 levels were doubled with leptin treatment. Although the mechanisms are still being explored, an augmented thyroid function might be a contributing factor to the significantly facilitated energy metabolism. A recent study demonstrates that leptin obviates the fasting induced decreases in plasma T3 and T4 levels and hypothalamic pro-thyrotropin-releasing hormone mRNA expression, suggesting a role of leptin in modulating the hypothalamus-pituitary-thyroid axis through which leptin participates in energy metabolism (28).

In summary, leptin treatment in the insulin-deficient diabetic rats restored normoglycemia, minimized body weight loss due to food restriction, substantially improved glucose metabolic rates during the postabsorptive state, and restored insulin sensitivities at the levels of both the liver and the peripheral tissues during glucose clamp. The effects on glucose turnover are largely independent of food restriction and changes in blood glucose concentration. Taking together, all these results point to a very potent antidiabetic effect of leptin. As these improvements take place in an insulin-deficient model of diabetes, we propose that leptin may be of clinical potential in helping treat human type 1 diabetes.

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