Central leptin increases insulin sensitivity in streptozotocin-induced diabetic rats

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Lin, Chia-Yu, D. Allan Higginbotham, Robert L. Judd, and B. Douglas White. Central leptin increases insulin sensitivity in streptozotocin-induced diabetic rats. Am J Physiol Endocrinol Metab 282:E1084–E1091, 2002; 10.1152/ajpendo.00489.2001.—This study examined the effect of intracerebroventricular leptin on insulin sensitivity in streptozotocin (STZ)-induced diabetic rats. Male Wistar rats were cannulated in the lateral ventricle and, after recovery, administered either intravenous STZ (50 mg/kg) to induce diabetes or citrate buffer. Chronic leptin (10 μg/10 μl icv) or vehicle injections were administered daily for 14 days beginning 2 days after establishment of hyperglycemia in the diabetic animals. At the end of the 2 wk of injections, insulin sensitivity was measured by the steady-state plasma glucose (SSPG) method. Blood glucose concentrations were dramatically reduced and normalized by the 4th day in diabetic animals receiving intracerebroventricular leptin treatment. Diabetic animals exhibited insulin resistance, whereas intracerebroventricular leptin significantly enhanced insulin sensitivity, as indicated by decreased SSPG. Circulating leptin levels were not increased in animals injected with intracerebroventricular leptin. Thus the increased peripheral insulin sensitivity appears to be due solely to the presence of leptin in the brain, not to leptin acting peripherally. These data imply that inadequate central leptin signaling may lead to insulin resistance.

intracerebroventricular leptin; insulin resistance; hyperglycemia

The interaction between leptin and insulin has been the subject of several investigations. Leptin is thought to be a signal that informs the brain about the size of the fat mass in the body. It acts as a satiety factor, decreasing food intake and increasing energy expenditure, or at least preventing the decrease in energy expenditure normally associated with a decrease in food intake (38). These effects lead to a decrease in body fat. In addition to these actions, leptin treatment enhances insulin sensitivity in normal rats, as indicated by increased insulin-stimulated glucose utilization in peripheral tissues (7, 33, 42). It also decreases plasma glucose and/or insulin concentrations of normal animals in the post-absorptive state. Leptin has been shown to directly inhibit insulin secretion (11), whereas insulin increases leptin release from adipocytes (25). Evidence indicates that glucose metabolism, rather than insulin itself, is the main determinant for leptin expression in adipose tissue (22). Moreover, in vivo and in vitro evidence suggests that leptin and insulin-signaling networks may be connected at several levels, such as insulin receptor substrates, or IRS; phosphatidylinositol 3-kinase; and mitogen-activated protein kinase, or MAPK (19, 20, 41). Therefore, leptin and insulin-signaling pathways may interact with each other.

The effects of leptin on insulin sensitivity have been examined independently of peripheral insulin concentrations. Diabetic animals induced by streptozotocin (STZ), which selectively destroys insulin-producing β-cells of the pancreas, are characterized by both insulin deficiency and insulin resistance (8), as well as low plasma leptin concentrations (17). Chinookoswong et al. (6) showed that the hyperglycemia in STZ-induced diabetic rats is normalized by chronic peripheral leptin injections independently of the effect of decreased food intake. Whereas untreated diabetic rats display insulin resistance, insulin sensitivity of STZ-induced diabetic rats is enhanced after chronic peripheral leptin administration more profoundly than by restoration of euglycemia or decreased food intake alone. The authors concluded that high levels of peripheral leptin act through insulin-independent and insulin-sensitizing mechanisms to modulate glucose metabolism in insulin-deficient diabetic rats. It is possible that peripheral leptin mimics and augments insulin signaling by increasing PI 3-kinase activity in insulin target tissues (19, 20) to enhance peripheral insulin action.

An association between insulin and leptin has been further implicated in some animal models of obesity. Obese mice (ob/ob), which lack functional leptin, are insulin resistant and develop diabetes (24). However, diabetic mice (db/db) and fatty rats (fa/fa) are also insulin resistant although they have elevated levels of circulating leptin. These rodents have a defect in the long form of the leptin receptor and are resistant to the effects of leptin (14). The long form of the leptin receptor is found at high levels in the ventromedial hypothalamus, an area thought to be an important site of...
leptin action (18, 33). These results suggest that it is unlikely that low levels of circulating leptin in the periphery are the major factor responsible for the induction of insulin resistance. It is more likely that a decrease in leptin signaling in the brain is related to insulin resistance.

To investigate the effects of central leptin on insulin sensitivity in diabetes, intracerebroventricular leptin was chronically administered to STZ-induced diabetic rats. The impact of brain leptin on peripheral insulin sensitivity was evaluated by the steady-state plasma glucose (SSPG) method. The results of this study suggest that leptin need only act centrally to increase peripheral insulin sensitivity. This implies that inadequate central leptin signaling may promote or lead to peripheral insulin resistance.

MATERIALS AND METHODS

Animals. Male Wistar rats (150–170 g; Harlan, Indianapolis, IN) were housed individually in hanging wire cages with a 12:12-h light-dark cycle (lights on 6 AM) in a temperature-controlled room. The animals were fed powdered laboratory rodent chow (Prolab RMH 3000 Meal, Purina Mills, Richmond, IN) and had free access to tap water. Experimental protocols were approved by Auburn University’s Institutional Animal Care and Use Committee before initiation of the experiments.

Experimental design. Nineteen rats were implanted with an intracerebroventricular cannula directed to the lateral ventricle (see Cannula placement). After recovery, approximately one-half of the rats were administered STZ via the tail vein to induce diabetes (see Induction of diabetes). The remainder of the animals received an injection of vehicle as a control. After diabetes was established, approximately one-half of the rats in each group (diabetic vs. control) received a daily injection of leptin (10 μg/10 μl icv, Calbiochem, San Diego, CA). The other rats received a daily intracerebroventricular injection of vehicle (PBS). Both leptin and vehicle were prepared according to the manufacturer’s instructions and were adjusted to pH 7.4. The intracerebroventricular injections were given daily for 14 days. Thus there were four treatment groups: control-vehicle, control-leptin, diabetic-vehicle, and diabetic-leptin. Groups consisted of 4–5 animals each. Body weight and food intake were monitored daily throughout the study. Blood glucose concentrations, via the tail vein, were monitored daily for animals in the other three treatment groups. After 14 days of intracerebroventricular injection, animals were fasted 6–7 h before insulin sensitivity was assessed by the SSPG method. This method was originally developed by Reaven and colleagues (43, 44) and modified by Suga et al. (40). Briefly, rats were anesthetized with pentobarbital (50 mg/kg), and cannulas were implanted in the right jugular vein and carotid artery. In addition, a tracheotomy was performed to ease the rats’ breathing. The body temperature of anesthetized animals was maintained with a heating pad. To prevent clotting, cannulas were flushed with freshly heparinized (20 U/ml) saline. The jugular cannula was used to continuously infuse insulin (2.5 mU·kg⁻¹·min⁻¹; Humulin R, Eli Lilly, Indianapolis, IN), glucose (8 mg·kg⁻¹·min⁻¹), and somatostatin (0.5 μg/min; Sigma, St. Louis, MO) for 170 min at a rate of 5 ml/h. Blood samples were collected from the carotid cannula before the infusion was initiated and 150, 160, and 170 min after the start of the infusion. The mean blood glucose concentration of the 150-, 160-, and 170-min samples was used to determine the SSPG value. Blood samples were collected for plasma insulin and plasma leptin analyses.

Pair-feeding. On the basis of results from the study just described, a second study was performed to determine whether the effects of leptin on serum glucose concentrations were mediated secondarily through changes in food intake. The design of this study was similar to the first except that all rats were treated with STZ, and a group of rats pair-fed to the level of the leptin-treated rats was also examined. Pair-fed animals received a daily intracerebroventricular injection of vehicle. Pair-feeding was performed by assigning each pair-fed rat to a partner in the leptin treatment group. The amount of food provided to each pair-fed animal was equal to the amount consumed by its leptin-treated partner during the previous 24-h period. To prevent prolonged fasting in the pair-fed animals, food was presented during three intervals throughout the day. One-quarter of the daily food was available between 5:00 AM and 6:00 AM, one-quarter was available between 11:00 AM and 6:00 PM, and one-half was available between 6:00 PM and 5:00 AM. Food cups were removed from all animals at 6:00 AM. Blood glucose concentrations were determined daily at 10:00 AM, and food was returned thereafter at ~11:00 AM. Therefore, blood glucose concentrations were monitored daily after 4 h of fasting for all animals. Intracerebroventricular injections of leptin (10 μg/10 μl) or vehicle were administered for 7 days.

Cannula placement. Animals were anesthetized by intraperitoneal injection of ketamine-xylazine (100 mg/kg ketamine and 1 mg/kg xylazine) and placed in a stereotaxic apparatus. A 22-gauge, stainless steel guide cannula (Plastic One, Roanoke, VA) was implanted into the lateral ventricle by use of the following coordinates: 0.8 mm posterior to bregma, 1.4 mm lateral to the midline, and 3.5 mm ventral to the surface of the skull. The guide cannula was secured to the skull with four stainless steel screws and dental cement. A removable “dummy” cannula that extended 1 mm beyond the guide cannula was inserted to prevent clogging of the guide cannula. The animals were placed in individual cages and allowed to recover for 4 days. Cannula placement was confirmed by a positive drinking response after administration of angiotensin II (40 ng/6 μl). Animals that did not drink ≥5 ml of water within 15 min after treatment were not included in the experiment. After the drinking test, 17 of 19 rats were used in this study.

Induction of diabetes. Insulin-deficient diabetes was induced with a single intravenous injection, via the tail vein, of freshly prepared STZ (50 mg/kg; Sigma) in 0.05 M citrate buffer (pH 4.5). This regimen produced plasma insulin levels of ~0.5 ng/ml and blood glucose >360 mg/dl in all treated animals in this experiment. Control animals received an injection of citrate buffer only.

Hormone and metabolite analysis. Plasma insulin was measured by a sensitive radioimmunoassay specific for rat insulin (Linco Research, St. Charles, MO), with a lower limit of detection of 0.02 ng/ml. Plasma leptin was measured by a radioimmunoassay for rat leptin (Linco Research). The limit of detection for this assay is 1.0 ng/ml. Blood glucose was determined by an Accu-Chek simplicity glucometer (Boehringer Mannheim, Indianapolis, IN).

Statistical analysis. Results are presented as means ± SE. Statistical analyses were performed by utilizing StatView software (SAS Institute, Cary, NC). Daily food intake and body weight data were evaluated by two-way analysis of variance (ANOVA) with repeated measures, with examination of the main effects of STZ and leptin treatments. In the second study, blood glucose concentrations were analyzed by
Table 1. Basal body weight, food intake, and blood glucose

<table>
<thead>
<tr>
<th></th>
<th>Control-Vehicle (n = 4)</th>
<th>Control-Leptin (n = 4)</th>
<th>Diabetic-Vehicle (n = 4)</th>
<th>Diabetic-Leptin (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>305.0 ± 4.8</td>
<td>301.5 ± 6.6</td>
<td>308.5 ± 7.4</td>
<td>301.6 ± 5.3</td>
</tr>
<tr>
<td>Food intake, g</td>
<td>26.0 ± 1.2</td>
<td>25.0 ± 1.2</td>
<td>26.1 ± 1.3</td>
<td>26.1 ± 0.6</td>
</tr>
<tr>
<td>Blood glucose, mg/dl</td>
<td>105.3 ± 2.5</td>
<td>100.8 ± 3.1</td>
<td>421.8 ± 41.8*</td>
<td>406.4 ± 25.2*</td>
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Values are means ± SE. See Experimental design in text for description of 4 treatment groups. Basal body weight and food intake were measured on the day immediately before streptozotocin (STZ) (50 mg/kg iv) or vehicle injection. Basal blood glucose concentrations were measured 2 days after STZ injection and immediately before leptin (10 μg/10 μl icv) or vehicle injection. *P < 0.0001 vs. control.

RESULTS

Body weight and food intake. Basal body weight and food intake were determined on the day immediately before STZ or citrate buffer injection. There were no differences in basal body weight and food intake among groups (Table 1). Body weight of control vehicle-treated animals increased gradually (5 g/day) after the 2nd day of the intracerebroventricular injection (Fig. 1A). The other groups did not show any weight gain throughout the experiment. Overall, STZ treatment or treatment of control and diabetic rats with leptin caused a decrease in body weight (Fig. 1A, F1,13 = 21.7, P < 0.0005 and F1,13 = 21.3, P < 0.0005, respectively). There was also a significant decrease in body weight with time after either leptin or STZ treatment (Fig. 1A, F1,143 = 18.8, P < 0.0001 and F1,143 = 4.5, P < 0.0001, respectively). The effects of leptin on body weight reduction were greater in controls than in diabetic animals during the study, resulting in a significant interaction between leptin and STZ (Fig. 1A, F1,143 = 13.9, P < 0.0001).

All groups except the diabetic vehicle-treated groups decreased food intake upon initiation of intracerebroventricular injection. Food intake of the control vehicle-treated rats recovered over the next few days to the level of intake before injections. Leptin treatment overall caused a decrease in food intake (Fig. 1B, F1,13 = 154.5, P < 0.0001). Leptin reduced daily food intake in both diabetic and control animals to ~20 g/day at the end of study. Food intake was increased significantly over time (Fig. 1B, F1,143 = 8.1, P < 0.0001), but leptin treatment attenuated this increase (Fig. 1B, F1,143 = 3.1, P = 0.001). The induction of diabetes led to an increase in food intake (Fig. 1B, F1,13 = 35.1, P < 0.0001), and there was an interaction between leptin and STZ treatment overall (Fig. 1B, F1,13 = 9.8, P < 0.01). The effects of leptin on food intake reduction were greater in diabetic than in control animals overall. However, the diabetic vehicle-treated rats increased their food intake by 36% compared with the control vehicle-treated animals (~34 vs. ~25 g/day). These diabetic animals

Fig. 1. Effect of icv leptin (10 μg/10 μl) or vehicle on body weight (A) and food intake (B) in control and diabetic rats. Values are means ± SE (n = 4 or 5). Day −2 refers to the day of streptozotocin (STZ) injection; day 0 refers to the starting day for leptin injection. There was a significant difference in food intake between control and diabetic rats after 6 and 7 days of icv leptin injection. *P < 0.005; †P < 0.05. Refer to RESULTS for differences in 4 treatment groups.
imals exhibited hyperphagia throughout the study. During the intracerebroventricular leptin injection, diabetic leptin-treated animals showed a decrease of ~45% in food intake compared with diabetic vehicle-treated animals. Leptin caused a 35% reduction of food intake in control animals overall. Food intake was much lower in control than in diabetic animals on days 6 and 7 after leptin treatment (P < 0.05). There was no difference in food intake between diabetic leptin-treated and control leptin-treated animals after this time.

**Diabetic leptin-treated blood glucose concentrations and fed vs. fasted blood glucose concentrations.** Basal blood glucose level was determined 2 days after STZ or citrate buffer injection. Basal blood glucose levels from diabetic animals were higher than those of control rats (Table 1, 413.2 ± 21.8 vs. 103 ± 2.0 mg/dl). Blood glucose levels from diabetic leptin-treated animals decreased dramatically after the third day of leptin injection (Fig. 2, P < 0.01). They continued to decrease and reached a normal blood glucose concentration (132.5 ± 14.2 mg/dl) by day 4 and remained normal throughout the remainder of the study. We compared fed and fasting blood glucose concentrations by averaging blood glucose over the last 4 days of injection as the fed-state blood glucose concentrations and compared them to values for blood glucose obtained after 6 h of fasting (Fig. 3). There were significant differences in the blood glucose levels between the fed and the fasted state for leptin-treated animals, both for control and diabetic animals (F1,4 = 132.2, P = 0.0005 and F1,6 = 17.5, P < 0.006, respectively). In these leptin-treated groups, fasting blood glucose concentrations were significantly lower than glucose concentrations in the fed state. In vehicle-treated rats, glucose concentrations in the fasted and fed states were nearly identical.

**Fasting plasma insulin, leptin, and glucose concentrations and SSPG levels.** Fasting blood glucose levels remained higher in diabetic vehicle-treated rats than in the other groups (Fig. 4A, P < 0.0001). Chronic central infusion of leptin markedly lowered fasting blood glucose in both diabetic and normal animals, and there was no difference between them. Control vehicle-treated rats maintained a normal concentration of blood glucose that was higher than that of leptin-treated animals (P < 0.02). Basal fasting plasma insulin levels were attenuated in diabetic vehicle-treated rats compared with control vehicle-treated rats (Fig. 5, 0.41 ± 0.08 vs. 2.04 ± 0.42 ng/ml, P < 0.05). Leptin treatment significantly reduced fasting insulin concentration in control rats (0.50 ± 0.41 ng/ml). No differences were found in fasting plasma insulin concentrations among control leptin-treated, diabetic vehicle-treated, and diabetic leptin-treated animals. The SSPG values, an indicator of insulin resistance obtained from the last 20 min of infusion, were significantly elevated from diabetic vehicle-treated animals compared with control vehicle-treated rats (Fig. 4B, 368 ± 51 vs. 188 ± 26 mg/dl, P < 0.005), indicating that diabetic animals were insulin resistant. Leptin treatment in either diabetic or control animals significantly decreased SSPG values (Fig. 4B, 29 ± 3 and 29 ± 2 mg/dl, respectively) compared with their respective controls, indicating that central leptin administration greatly increased peripheral insulin sensitivity. There was a significant correlation between fasting blood glucose concentrations and the SSPG value (Fig. 4C, r = 0.95, P < 0.0001). Except for the control vehicle-treated group, plasma leptin concentrations after fasting and during SSPG were below the detection limit of the assay (1 ng/ml) (data not shown).

**Fasting blood glucose concentrations in pair-fed rats.** Fasting glucose concentrations in rats pair-fed to the level of rats treated with leptin were not significantly different from those of rats treated with vehicle (Fig. 6). Similar to the data in Fig. 2, intracerebroventricular leptin injections to diabetic rats resulted in a decrease in blood glucose concentration. By the 4th day of leptin treatment, blood glucose concentrations had re-
turned to normal levels. Over the treatment period, glucose concentrations were lower in leptin-treated rats than in pair-fed (\(P < 0.003\)) or vehicle-treated rats (\(P < 0.005\)).

**DISCUSSION**

In the present study, we assessed the role of central leptin on peripheral insulin sensitivity. To exclude the possible interference of peripheral leptin and insulin, we induced diabetes in rats with STZ, a drug selectively destroys insulin-producing \(\beta\)-cells of the pancreas, providing a model of type 1 diabetes. Because peripheral insulin action or glucose uptake is required for endogenous leptin release from fat cells (22), diabetic rats also have low peripheral leptin concentrations (17). In this study, we have demonstrated that chronic central leptin administration at levels that do not alter peripheral leptin concentration greatly increased peripheral insulin sensitivity and normalized blood glucose levels in diabetic rats.

These findings extend the work of Chinookoswong et al. (6), who showed that high doses (4 mg·kg\(^{-1}\)·day\(^{-1}\)) of peripheral leptin for 2 wk normalize blood glucose concentrations and increase insulin sensitivity in STZ-treated diabetic rats. The enhanced peripheral insulin sensitivity occurs without the presence of adequate amounts of insulin in the periphery after high concentration of peripheral leptin administration in diabetic rats. Although the present study does not exclude a peripheral action of leptin, it does suggest that only the central actions of leptin are required to increase insulin sensitivity and normalize blood glucose concentra-

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**Fig. 4.** Fasting glucose (A) and SSPG (B) concentrations of control and diabetic rats with either icv leptin (10 \(\mu\)g/10 \(\mu\)l) or vehicle injection. Values are means ± SE (n = 3 or 4). For fasting blood glucose concentration (A), there was a significant decrease in blood glucose concentrations after icv leptin treatment. \(*P < 0.0001\) vs. Diabetic-Vehicle; \(\ddagger P < 0.02\) vs. Control-Leptin and Diabetic-Leptin; \(\ddagger\ddagger P < 0.0001\) vs. Diabetic-Vehicle. For the SSPG (B), insulin sensitivity was significantly increased after icv leptin treatment in both control and diabetic rats. \(\ddagger P < 0.001\) vs. Diabetic-Leptin and Diabetic-Vehicle. For the SSPG (B), insulin sensitivity was significantly increased after icv leptin treatment in both control and diabetic rats. \(\ddagger P < 0.001\) vs. Diabetic-Leptin and Diabetic-Vehicle. Fasting blood glucose level (BG) was highly correlated with SSPG (C) (\(r = 0.951, P < 0.0001\)).

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**Fig. 5.** Fasting plasma insulin of control and diabetic rats with either icv leptin (10 \(\mu\)g/10 \(\mu\)l) or vehicle injection. Values are means ± SE (n = 3–5). Plasma insulin concentration was significantly greater in Control-Vehicle animals than in Control-Leptin and Diabetic-Vehicle animals. \(\ddagger P < 0.05\) vs. Control Vehicle.

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**Fig. 6.** Daily blood glucose concentrations among leptin-treated (10 \(\mu\)g/10 \(\mu\)l), vehicle-treated, and pair-fed rats. Pair-fed rats were vehicle treated and food restricted to the level of leptin-treated rats. Values are means ± SE. Day 0, 1st day of icv leptin or vehicle injection. Blood glucose concentrations were significantly decreased 1 day after icv leptin treatment (\(\ddagger P < 0.00005\)) and were normalized after 4 days.
tions. Similar findings by Chinookoswong et al. (5) have been presented in abstract form. Together, these studies suggest that leptin acting centrally can enhance peripheral sensitivity to insulin independently of normal circulating concentrations of insulin and leptin. These data are consistent with studies that have found leptin, administered either centrally or peripherally, to increase insulin-stimulated glucose utilization in peripheral tissues in normal (nondiabetic) rats (2, 7, 33, 37, 42). Interestingly, Rossetti’s group (Obici et al., Ref. 23) has recently published results complementary to the present findings. They demonstrated that chronic central α-melanocyte-stimulating hormone (α-MSH) administration increases insulin sensitivity during insulin clamp studies. The rate of glucose infusion required to maintain plasma glucose concentration is increased by 56% with α-MSH and decreased by 32% with the melanocortin receptor antagonist SHU 9119. The effects of α-MSH are associated with an increase in the phosphorylation of IRS-1 and IRS-2 in the liver. Because leptin is known to activate the central melanocortin system (31, 32), these findings suggest that central leptin acts through the melanocortin system to increase insulin sensitivity.

It is possible that blood glucose concentrations were normalized in diabetic leptin-treated rats by the increase in peripheral insulin sensitivity, as demonstrated by the reduction in the SSPG concentrations. Although STZ treatment did substantially decrease plasma insulin concentrations, they were still at measurable levels. The increased sensitivity to insulin after central leptin injection could have more than compensated for the decrease in insulin concentration, resulting in a normalization of blood glucose concentration in diabetic rats. In the present study, fasting blood glucose levels after central leptin treatment were lower than those of control animals, despite the reduction in postabsorptive insulin concentration after leptin treatment. This also suggests an increase in peripheral insulin sensitivity. However, the cause and effect relationship between blood glucose concentration and insulin sensitivity is not completely understood. Although insulin sensitivity can affect blood glucose concentrations, there is also evidence that the blood glucose concentration can affect insulin sensitivity (26, 27). Therefore, it is also possible that chronic central leptin administration lowered the concentration of blood glucose, which in turn increased the sensitivity to insulin.

Direct measurement of nerve activity has suggested that leptin increases sympathetic activity to several tissues. Haque et al. (15) suggested that the leptin-induced activation of the sympathetic nervous system and insulin act cooperatively with regard to glucose homeostasis, because central leptin increases glucose uptake to a greater degree than insulin alone. The synergistic action of the two factors on glucose uptake is dependent on intact sympathetic innervation of tissues. Sympathetic nerves may cross-talk with the insulin-signaling pathways to improve glucose uptake in response to insulin in some tissues. Thus the effect of central leptin to alter glucose homeostasis may be mediated via the sympathetic nervous system.

Chronic central leptin infusion resulted in markedly decreased food intake and body weight in this study. Therefore, it was possible that the attenuation in blood glucose concentration of diabetic leptin-treated rats could have resulted secondarily from the decreased food intake. However, this was not supported by the data from rats pair-fed to the level of the leptin-treated rats. Blood glucose concentrations of pair-fed rats remained greater than those of leptin-treated rats and were not significantly different from those of vehicle-treated rats. This suggests that central leptin has effects on glucose homeostasis that are independent of its ability to decrease food intake. Chinookoswong et al. (5) showed similar results in a published abstract. Therefore, the dramatic effects of central leptin to normalize blood glucose concentrations and increase insulin sensitivity in diabetic rats cannot be fully accounted for by the decrease in food intake.

Interestingly, food intake did appear to interact with blood glucose concentration in rats that were treated with leptin. Leptin treatment in both control and diabetic rats resulted in a significantly lower blood glucose concentration when animals were fasting compared with the blood glucose concentration taken when animals had access to food. This was not observed for rats receiving vehicle injections. The significance of this is not clear at the present time, but the results suggest that leptin-treated rats may need to eat during the morning hours (a time when rats normally do not eat very much) to help maintain their glucose concentrations.

In this study, as expected, diabetic rats lost weight even though they were hyperphagic, consistent with previous studies (6, 16, 17, 34, 35). Leptin treatment in these diabetic animals reversed the increased food intake to a level lower than that of control vehicle-treated animals but similar to that of control leptin-treated animals. Therefore, leptin treatment caused a greater drop in food intake in diabetic than in control rats. At the same time, leptin seems to prevent a drop in body weight in diabetic rats. Leptin may increase utilization of energy more efficiently in diabetic animals, and this may be due to the enhanced anabolic action of insulin in peripheral tissues. Moreover, diabetic animals with leptin treatment had a higher food intake than control leptin-treated rats after 6–8 days of leptin injection. The difference in food intake between diabetic leptin-treated and control leptin-treated rats may be due to a lack of insulin signal in the brain in the diabetic rats. Both insulin and leptin have been shown to act via the central nervous system to inhibit feeding (9, 12, 39). Therefore, food intake may be stimulated by a decrease in the circulating level of either leptin or insulin. Diabetic hyperphagia is suppressed by administration of insulin directly into the brain (35) or by subcutaneous insulin implant (17). Subcutaneous leptin infusion at a rate that prevents circulating leptin from decreasing (34) or induces hy-
perleptinemia (6) in STZ diabetic rats also markedly inhibits food intake. Although insulin is the predominant controller of blood glucose levels, recent studies have indicated that brain leptin status is important in plasma glucose regulation and insulin sensitivity. It has been reported that the absence of leptin or of a functional leptin receptor causes massive obesity and non-insulin-dependent diabetes mellitus (13, 21, 45). Insulin resistance is a well known feature in human obesity and in db/db mice, which are leptin resistant, and in ob/ob mice, which lack endogenous functional leptin. Although direct evidence for a relationship between increased body fat and insulin resistance exists, conditions that decrease body fat, such as lipodystrophy, also cause insulin resistance. Hyperleptinemia via transgenic overexpression of leptin in lipoatrophic diabetic mice reverses insulin resistance (10). In the present study, diabetic animals with insulin and leptin deficiency had no visible fat and were insulin resistant. Leptin appears to be transported across the blood-brain barrier by a saturable system (1). Thus leptin resistance could occur at the level of transportation of leptin into the brain (4, 32). Alternatively, resistance could occur at a postreceptor level (28). Either through leptin resistance or leptin deficiency, the central leptin signaling can become inadequate. In this study, STZ-diabetes with sufficient STZ dosing resulted in marked insulin deficiency. Plasma leptin levels were also reduced, as shown in previous studies (3, 17, 34, 36). Plasma leptin levels were below the detection limit of assay for diabetic animals and animals with central leptin treatment. Central leptin dramatically increased insulin sensitivity in diabetic animals without apparently altering peripheral leptin concentrations, indicating that there was no leakage of the hormone into the circulation. Altogether, this study suggests that inadequate central leptin signaling leads to insulin resistance.

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