Chronic central leptin infusion restores hyperglycemia independent of food intake and insulin level in streptozotocin-induced diabetic rats

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ABSTRACT **We examined the effects of chronic centrally administered leptin on the glucose metabolism of streptozotocin-induced diabetic (STZ-D) rats, a model for insulin-dependent diabetes mellitus. When 3 μg · rat^{-1} · day^{-1} of leptin was infused into the third ventricle for 6 consecutive days (STZ-LEP), STZ-D rats became completely euglycemic. The effect was not seen when the same dosage was administered s.c. Centrally administered leptin did not affect peripheral insulin levels. The feeding volume of STZ-LEP rats was suppressed to the level of non-STZ-D control rats. No improvement of hyperglycemia was noted when STZ-D rats were pair-fed to match the feeding volume of STZ-LEP rats. Thus, the euglycemia of STZ-LEP rats cannot be due to the decreased feeding volume. In the STZ-D rat, glucokinase mRNA, a marker of glycolysis, is down-regulated whereas glucose-6-phosphatase mRNA, a marker of gluconeogenesis, and glucose transporter (GLUT) 2, which is implicated in the release of glucose from liver, are up-regulated. GLUT4, uncoupling protein (UCP) 1, and UCP3 were down-regulated in brown adipose tissue. These parameters returned to normal upon central infusion of leptin. GLUT4 was not down-regulated in the skeletal muscle of STZ-D rats; however, fatty acid binding protein and carnitine palmitoyltransferase I, markers for utilization and β-oxidation of fatty acids, were up-regulated and restored when the rats were treated with leptin. The increase and subsequent decrease of fatty acid utilization suggests a decrease of glucose uptake in the skeletal muscle of STZ-D rats, which was restored upon central leptin administration. We conclude that centrally infused leptin does not control serum glucose by regulating feeding volume or elevating peripheral insulin, but by regulating hepatic glucose production, peripheral glucose uptake, and energy expenditure. The present study indicates the possibility of future development of a new class of anti-diabetic agents that act centrally and independent of insulin action.—Hidaka, S., Yoshimatsu, H., Kondou, S., Tsuruta, Y., Oka, K., Noguchi, H., Okamoto, K., Sakino, H., Teshima, Y., Okeda, T., Sakata, T. Chronic central leptin infusion restores hyperglycemia independent of food intake and insulin level in streptozotocin-induced diabetic rats. *FASEB J.* 16, 509–518 (2002)**

Key Words: STZ-induced insulin-deficient diabetes · glucose transporters · uncoupling proteins · anti-diabetic drugs

LEPTIN IS A circulating satiety factor produced by the adipocyte whose expression reflects the body's state of nutritional reserves (1). Leptin plays an important role in regulating energy stores and in the choice of fuels to be used under various nutritional conditions (2). Evidence indicating that leptin may play especially important roles in the regulation of glucose homeostasis has accumulated recently (1–3). In vitro findings indicate that leptin acts directly on specific peripheral cells such as hepatocytes, islet cells, adipocytes, and skeletal muscle cells (4–7). Several in vivo studies have shown that systemic administration of leptin not only enhances glucose turnover in normal rodents (8–10), but also ameliorates impaired glucose metabolism in leptin-deficient *ob/ob* mice (11) and severe insulin-resistant lipodystrophic mice (12, 13). High-dose systemic administration of leptin improves hyperglycemia in insulin-deficient streptozotocin (STZ) -induced diabetic (STZ-D) rats (14).

Several lines of evidence suggest that the central nervous system (CNS) may be a critical target for leptin action by which it maintains euglycemia without affecting insulin secretion (8, 15–18). Many of the anti-diabetic effects observed after peripheral administration of leptin are likely due to the interaction of leptin with receptors in the brain. It is important to investigate whether the anti-diabetic effects of leptin are mediated through the CNS, because the hypothalamus has been shown to be the main target of leptin action in controlling feeding behavior and energy metabolism.

We investigated the effects of chronic central administration of leptin on changes in glucose, insulin, and

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related parameters that affect glucose metabolism, energy expenditure, fatty acid utilization, and β-oxidation under diabetic conditions. Leptin was slowly infused over a course of 6 days into the third ventricle (i3vt) of the brain in rats confirmed to be deficient in insulin.

The objective of the study was to clarify the therapeutic implications of centrally administered leptin on the insulin-deficient diabetic state. Although direct infusion of leptin into the brain may not be practical in humans, agents to enhance central leptin action or the transport of leptin through the blood–brain barrier, even those that elevate neurochemical reactions downstream of leptin, may be feasible as potential treatments for diabetes if centrally administered leptin is shown to improve serum glucose levels.

MATERIALS AND METHODS

Research design

Twenty-five animals were divided into five groups of five rats each: *1)* a citrate buffer control group infused i3vt with saline (CIT-SAL); *2)* a STZ-D group infused i3vt with saline (STZ-SAL); *3)* a STZ-D group infused i3vt with leptin (STZ-LEP); *4)* a STZ-D group pair-fed with STZ-LEP group (STZ-PF); and *5)* a STZ-D group injected with leptin subcutaneously (s.c. or sc) (STZ-LEPsc). The STZ-PF group, with matched food intake to STZ-LEP rats, was introduced to exclude the secondary effect of leptin-induced feeding suppression. The STZ-LEPsc group excluded the effect of leptin leakage into systemic circulation on peripheral glucose metabolism. The procedures in the STZ-PF and STZ-LEPsc groups were the same as for the STZ-SAL and STZ-LEP groups, as applicable. Twelve animals were divided into three groups of four rats each: *1)* a CIT-SAL group; *2)* a nondiabetic CIT group infused i3vt with leptin (CIT-LEP); and *3)* a group of nondiabetic CIT controls pair-fed with the CIT-LEP group (CIT-PF). The three groups were set up to elucidate whether the effects of centrally infused leptin on the regulation of various genes in peripheral tissues were specific for insulin-deficient animals or were more generally applicable.

Serum concentrations of glucose, insulin, and leptin were quantified and Northern blot analysis was performed on mRNA of the rat glucose transporter (GLUT) 2, GLUT4, uncoupling protein (UCP) 1, UCP3, glucose-6-phosphatase (G-6-Pase), phosphoenolpyruvate carboxykinase (PEPCK), glucokinase (GK), lipoprotein lipase (LPL), hormone-sensitive lipase (HSL), carnitine palmitoyltransferase I (CPT I), and muscle-type fatty acid binding protein (M-FABP) from various organs extracted at the time of the animal's death. These RNA parameters were examined to see how the release and uptake of glucose, energy expenditure, fatty acid utilization, and β-oxidation in the peripheral organs were affected by STZ treatment and to what extent they recovered after leptin infusion.

Animals

The subjects were mature male Wistar King A rats, 13–14 wk of age. All animals were housed in a room illuminated daily from 0700 to 1900 h (a 12:12 h light/dark cycle) with temperature maintained at 21 ± 1°C and humidity at 55 ± 5%. Animals were allowed free access to tap water and standard pellet rat chow (CE-2, Clea Japan, Tokyo, Japan) ad libitum except for the pair-fed animals. The pair-fed groups were given rat chow matched with the mean daily food intake of the leptin-administered rats. One-third of the daily total amount was given at 0700 h; the remaining two-thirds were given just before the onset of the dark phase (1900 h). The feeding schedule was based on preliminary experiments on food consumption over the course of the day. The last feeding on the final experimental day was omitted because all the animals were killed at 0710–0720 h. All studies were conducted in accordance with the Oita Medical University Guidelines based on the NIH Guide for the Care and Use of Laboratory Animals.

Surgery for chronic implantation of infusion cannula i3vt and osmotic mini-pump

Under intraperitoneal anesthesia with 45 mg/kg sodium pentobarbital, each rat was fixed in a stereotaxic apparatus (Narishige, Tokyo, Japan) to be chronically implanted i3vt with a stainless steel guide cannula (23 gauge) at least 7 days before STZ infusion. A stainless steel wire stylet (29 gauge) was inserted in the guide cannula to prevent leakage of the cerebrospinal fluid and obstruction of the cannula. Details of the surgical procedures have been described elsewhere (19). On the 15th day after STZ injection, an osmotic minipump was implanted into the s.c. space connected to the i3vt cannula with a polyethylene catheter under ether anesthesia. In the nondiabetic control experiments to investigate the effects of leptin on gene regulation, an i3vt cannula was inserted at least 7 days before osmotic minipump implantation. The procedures in these experiments were the same as for the STZ-D experiments, as applicable.

Venous treatment with STZ and i3vt leptin infusion

A solution of STZ (Sigma, St. Louis, MO) was prepared fresh on the day of infusion by dissolving STZ in sterile sodium citrate buffer solution to a final concentration of 0.1 M (pH 4.5). Rats were made diabetic by injecting STZ (60 mg/kg) into the tail vein at 1850–1900 h under light ether anesthesia. The control group was injected with a citrate buffer solution containing no STZ. At the time of implantation of the osmotic mini-pump, STZ rats whose blood glucose concentration was >400 mg/dl in a fed state were defined as diabetic. Food intake of STZ rats was confirmed to be twofold greater than that of the controls. This hyperphagic response is consistent with previous reports (14, 20, 21).

Murine leptin (Pepro Tech, Ltd., St. James' Square, London, UK) was infused i3vt through an Alzet osmotic mini-pump (Alzet, Model 2001, Alza, Palo Alto, CA) at a dose of 3 $\mu g \cdot rat^{-1} \cdot day^{-1}$ for 6 days. An identical volume of saline was infused i3vt through an osmotic mini-pump. For the STZ-LEPsc group, an identical dose of leptin was infused into the s.c. space through a mini-pump. The infusion was started on the 15th day after STZ injection.

Blood sampling and tissue extraction

Blood samples were collected through a chronically indwelling silicone catheter implanted in the right external jugular vein with its end inside the superior vena cava at a point immediately anterior to the atrium. Details of the surgical procedures have been described elsewhere (22). Surgical catheterization was carried out under ether anesthesia at least 7 days before STZ injection. Blood samples were taken at 0710–0720 h immediately before dissection of tissue samples. Serum samples were frozen at −20°C until assay for humoral factors. The following tissues were surgically removed and

quickly frozen in liquid nitrogen: brown adipose tissue (BAT), skeletal muscle, and liver. All tissue samples were stored at −80°C until RNA extraction.

Assay of blood- and adipocyte-borne humoral factors

Serum concentrations of insulin and leptin were quantified using an insulin radioimmunoassay (RIA) kit (Rat insulin [^{125}I] assay system, Amersham-Pharmacia, Little Chalfont, UK) and a rat leptin RIA kit having 100% cross-reactivity with murine leptin (Linco, St. Louis, MO), respectively. Serum glucose was measured by commercially available kits (Merck-auto glucose, Kanto Chemical, Tokyo, Japan).

RNA extraction and Northern blot analysis

Total RNA was extracted using the standard acid guanidinium phenol-chloroform method (23). Details of Northern blot analysis are described elsewhere (23). Complementary DNA (cDNA) probes for rat GLUT2, GLUT4, UCP1, UCP3, G-6-Pase, PEPCK, GK, LPL, HSL, CPT I, and M-FABP were prepared by RT-polymerase chain reaction (PCR). Details of PCR conditions and all primer sequences are available upon request. Identification of the appropriately sized product was carried out by mapping with multiple restriction endonucleases and sequencing. The hybridization signals were analyzed with a BIO-image analyzer, BAS 2000 (Fuji Photo-film Co., Tokyo, Japan). Values were normalized to the signal obtained after stripping and reprobing the same membranes with labeled an 18S rRNA cDNA probe (24).

Statistical analysis

Data were expressed as means ± SE. Statistical significance was assessed by one-way analysis of variance, followed by Scheffe's multiple comparison test. A P value of 0.05 was used as the threshold for statistical significance.

RESULTS

Food intake increased with STZ treatment and returned to normal when leptin was centrally infused. Body weight gain was retarded in all STZ-treated rats regardless of leptin treatment

Figure 1*A* shows cumulative food intake for 6 days in each group. Food intake in STZ-SAL rats was more than twofold greater than in the CIT-SAL group ($P<0.0001$). The i3vt infusion of leptin in STZ-treated rats restored food intake to the level of the CIT-SAL group. The STZ-LEPsc group did not differ significantly in cumulative food intake from the STZ-SAL group.

As shown in Fig. 1*B*, the CIT-SAL group gained body weight normally during the 6-day infusion period. In contrast, body weights in both the STZ-SAL and STZ-LEP groups were retarded compared with the increase in body weight in the CIT-SAL group ($P<0.001$, $P<0.0001$, respectively). This body weight reduction was also observed in the STZ-LEPsc group ($P<0.001$). Body weight reduction in the STZ-PF group was greater than that seen in the three remaining groups treated with STZ ($P<0.0001$; for each).

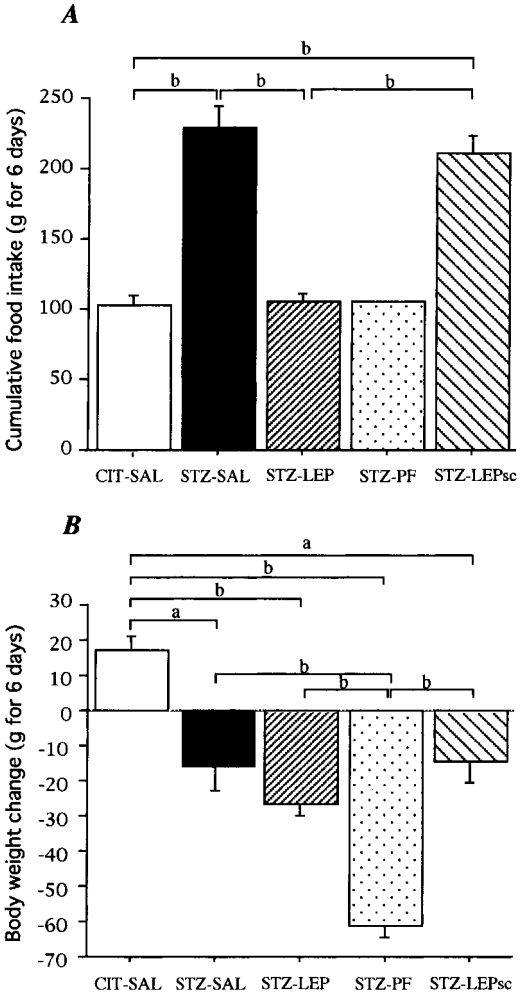

Figure 1. Effects of chronic i3vt infusion of leptin or saline on cumulative food intake (*A*) and body weight change from initial levels (*B*) in STZ-D rats. CIT-SAL, citrate buffer control group infused i3vt with saline; STZ-SAL, STZ-D group infused i3vt with saline; STZ-LEP, STZ-D group infused i3vt with leptin; STZ-PF, STZ-D group pair-fed with STZ-LEP group; and STZ-LEPsc, STZ-D group injected with leptin s.c. $n = 5$ for each group. Values are means ± SE. a = $P < 0.001$, b = $P < 0.0001$.

Serum glucose was restored to normal levels with central leptin infusion. Serum insulin and leptin levels were unaffected

In STZ-SAL rats, the serum concentration of glucose was confirmed to be elevated (469±19 mg/dl) vs. that of the CIT-SAL group (85±3 mg/dl) ($P<0.0001$) (**Fig. 2*A***). However, hyperglycemia STZ-SAL was dramatically and completely restored to the level of CIT-SAL animals after chronic i3vt infusion of leptin, as shown in STZ-LEP rats (88±7 mg/dl) ($P<0.0001$) (Fig. 2*A*).

In contrast, hyperglycemia in both STZ-PF and STZ-LEPsc rats was not improved and was sustained, maintaining glucose levels similar to that of the STZ-SAL group (STZ-PF; 427±17 mg/dl, STZ-LEPsc; 457±22 mg/dl) (Fig. 2*A*). STZ treatment was shown to negate insulin in the STZ-SAL group (CIT-SAL vs. STZ-SAL; $P<0.0001$) (Fig. 2*B*). I3vt leptin infusion did

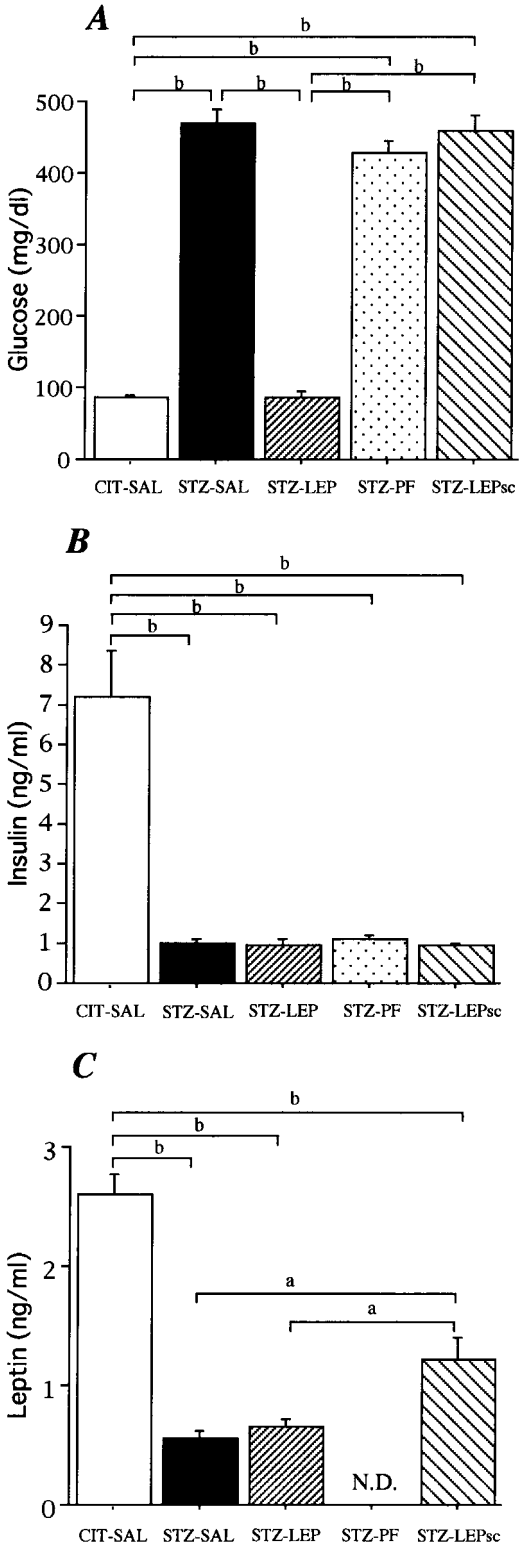

Figure 2. Effects of chronic i3vt infusion of leptin or saline on serum concentrations of glucose (*A*), insulin (*B*), and leptin (*C*) in STZ-D rats. Blood samples were taken immediately after the start of the light period on the final (6th) day. n = 5 for each group. Values are means ± SE. a = $P < 0.01$, b = $P < 0.0001$. N.D.; not detectable.

not affect serum insulin concentrations, which remained lower than those in the CIT-SAL group ($P<0.0001$) (Fig. 2*B*). Serum insulin concentrations remained lower in STZ-PF and STZ-LEPsc rats than the CIT-SAL group ($P<0.0001$; for each) (Fig. 2*B*). Serum leptin concentrations were decreased in all groups treated with STZ ($P<0.0001$ vs. CIT-SAL group for each) (Fig. 2*C*). When leptin was injected s.c., the serum leptin concentration was partially recovered, as shown in the STZ-LEPsc group vs. STZ-SAL and STZ-LEP rats ($P<0.01$; for each), although the value was not restored to control concentrations of the CIT-SAL group ($P<0.0001$) (Fig. 2*C*). The concentration was drastically decreased to an undetectable level when food intake was restricted to that of the STZ-LEP group, as demonstrated by the STZ-PF group (Fig. 2*C*).

Molecular parameters suggesting increased glucose production in liver were up-regulated in STZ treated rats, but dropped to normal levels when leptin was infused

Expression of GLUT2, G-6-Pase, GK, and PEPCK mRNAs is shown in **Fig. 3*A***. mRNA expression of GLUT2 ($P<0.0001$), G-6-Pase ($P<0.01$), and PEPCK ($P<0.01$) in the liver is up-regulated in STZ-SAL rats compared with the CIT-SAL control group. As in the STZ-LEP group, chronic i3vt leptin infusion restored up-regulation of GLUT2 and G-6-Pase mRNAs in STZ-treated rats to levels corresponding to those in the CIT-SAL control group. GLUT2 and G-6-Pase mRNA expression was affected slightly by pair-feeding (STZ-SAL vs. STZ-PF; $P<0.05$ in both parameters), but the effect was not as strong as seen with the STZ-LEP group ($P<0.001$ in GLUT2; $P<0.01$ in G-6-Pase). PEPCK mRNA was unaffected by the i3vt leptin infusion (STZ-SAL vs. STZ-LEP) or by pair-feeding (STZ-SAL vs. STZ-PF). In contrast to these three parameters, liver GK mRNA was down-regulated in STZ-SAL rats compared with CIT-SAL animals ($P<0.001$). The leptin infusion restored control levels of GK expression ($P<0.05$ vs. STZ-SAL), but did so incompletely ($P<0.05$ vs. CIT-SAL). Pair-feeding did not affect GK mRNA expression significantly (STZ-SAL vs. STZ-PF).

Molecular parameters indicative of glucose uptake in the periphery decreased with STZ treatment, but recovered upon central leptin infusion

Figure 3*B* shows GLUT4 mRNA levels in each group. STZ treatment down-regulated GLUT4 mRNA in BAT compared with the corresponding CIT-SAL control ($P<0.01$). The STZ-induced down-regulation was restored by leptin infusion ($P<0.05$) to such an extent that there remained no significant difference between the CIT-SAL and STZ-LEP groups. BAT GLUT4 mRNA in the STZ-PF group remained at the same level as the STZ-SAL group. On the other hand, GLUT4 mRNA in skeletal muscle was not affected by STZ treatment, leptin infusion, or pair-feeding.

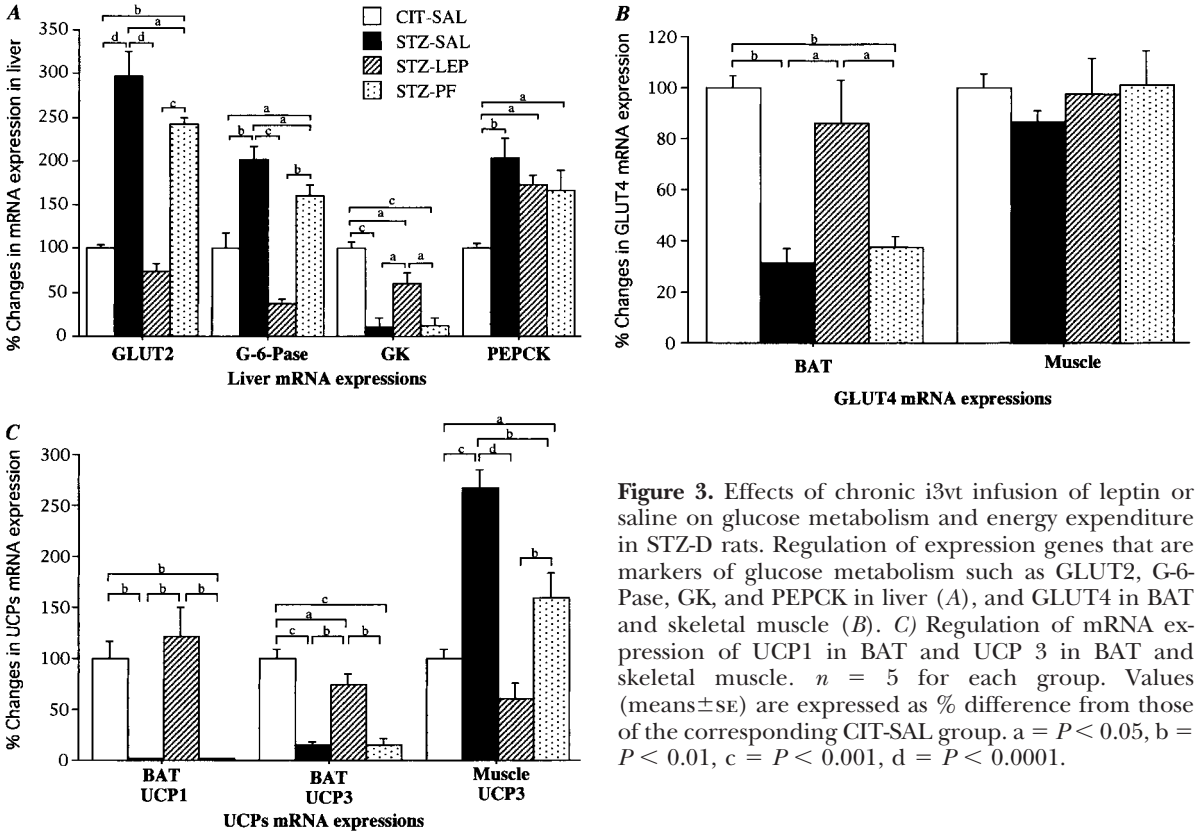

Figure 3. Effects of chronic i3vt infusion of leptin or saline on glucose metabolism and energy expenditure in STZ-D rats. Regulation of expression genes that are markers of glucose metabolism such as GLUT2, G-6-Pase, GK, and PEPCK in liver (*A*), and GLUT4 in BAT and skeletal muscle (*B*). *C)* Regulation of mRNA expression of UCP1 in BAT and UCP 3 in BAT and skeletal muscle. $n = 5$ for each group. Values (means±SE) are expressed as % difference from those of the corresponding CIT-SAL group. a = $P < 0.05$, b = $P < 0.01$, c = $P < 0.001$, d = $P < 0.0001$.

Molecular parameters indicative of heat production in the periphery were altered by STZ treatment but recovered, partially or completely, upon central leptin infusion

Figure 3*C* shows UCP gene expression in each group. UCP1 and UCP3 mRNAs in BAT were down-regulated in the STZ-SAL group compared with the CIT-SAL group ($P<0.01$, $P<0.001$, respectively), but UCP3 mRNA in skeletal muscle was up-regulated ($P<0.001$). I3vt leptin infusion restored the altered UCP mRNA expression to control levels partially or completely. BAT UCP1 rose and skeletal muscle UCP3 declined to levels not significantly different from the CIT-SAL group, whereas BAT UCP3 rose slightly but not to control levels (CIT-SAL vs. STZ-LEP; $P<0.05$). As shown in STZ-PF rats, pair-feeding did not affect BAT UCP1, or UCP3 mRNA compared with the STZ-SAL group; pair-feeding was slightly restorative of skeletal muscle UCP3 mRNA levels (STZ-SAL vs. STZ-PF; $P<0.01$) but recovery was much less complete than that achieved with central leptin supplementation (STZ-LEP vs. STZ-PF; $P<0.01$).

Molecular markers of peripheral fatty acid utilization was altered by STZ treatment but returned to normal, partially or completely, upon central leptin infusion

Figure 4*A* shows LPL, HSL, and CPT I mRNA levels in BAT for each group. Expression of LPL ($P<0.001$), HSL ($P<0.001$), and CPT I ($P<0.01$) in BAT was down-regulated in STZ-SAL rats compared with CIT-SAL controls. The i3vt leptin infusion restored mRNA expression to CIT-SAL control levels in the case of LPL and CPT I or partially for HSL (CIT-SAL vs. STZ-LEP; $P<0.01$). Pair-feeding did not affect BAT LPL, HSL, or CPT I mRNA (STZ-PF vs. STZ-SAL).

As shown in Fig. 4*B*, CPT I and M-FABP gene expression in skeletal muscle was up-regulated by STZ treatment vs. CIT-SAL controls ($P<0.001$, $P<0.01$, respectively). The effect was reversed by leptin infusion to levels not significantly different from those of the CIT-SAL group. Pair-feeding did not affect CPT I or M-FABP mRNA in skeletal muscle compared with the STZ-SAL group.

Peripheral gene expression after chronic i3vt infusion of leptin or pair-feeding in nondiabetic control rats

As shown in **Table 1**, leptin infusion up-regulated GLUT4 and UCP1 gene expression in BAT compared with the CIT-SAL group ($P<0.05$; for each). Pair-feeding did not affect BAT GLUT4 or UCP1 gene expression vs. the CIT-SAL group ($P<0.05$). Leptin infusion did not affect UCP3 mRNA expression compared with the CIT-SAL group, but the suppressive effect of pair-feeding on UCP3 mRNA was prevented by leptin infusion ($P<0.05$). No significant difference in GLUT4 mRNA was found in skeletal muscle among the three groups. In the liver, pair-feeding did not affect GLUT2, G-6-Pase, or PEPCK mRNA expression compared with CIT-SAL controls. Central leptin infusion down-regulated GLUT2 ($P<0.001$) and G-6-Pase

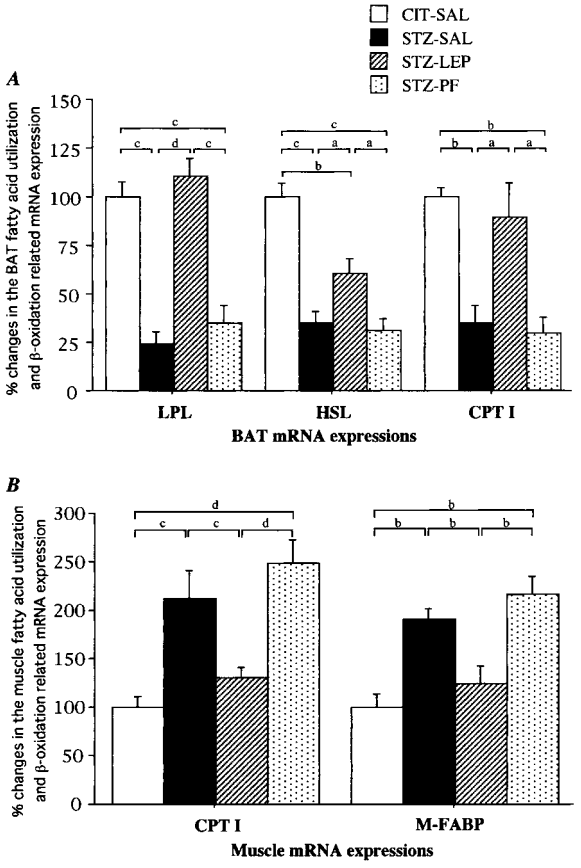

Figure 4. Effects of chronic i3vt infusion of leptin or saline on fatty acid utilization and β-oxidation in STZ-D rats. Regulation of expression of markers of fatty acid utilization and β-oxidation such as LPL, HSL, CPT I in BAT (*A*) and that of CPT I and M-FABP in skeletal muscle (*B*). $n = 5$ for each group. Values (means±SE) are expressed as % difference from those of the corresponding CIT-SAL group. a = $P < 0.05$, b = $P < 0.01$, c = $P < 0.001$, d = $P < 0.0001$.

($P<0.0001$) mRNA compared with the CIT-SAL group. Central leptin infusion did not affect PEPCK mRNA expression. Leptin infusion down-regulated GK mRNA compared with the CIT-SAL group ($P<0.001$), but the suppressive effect of pair-feeding on GK mRNA expression was prevented by leptin infusion ($P<0.0001$).

DISCUSSION

Centrally infused leptin induced euglycemia in insulin-deficient STZ-D rats without raising the serum level of insulin. Diabetic rats given a peripheral infusion of the same dose and those pair-fed to the centrally treated rats did not display significant improvements in blood glucose. From these results, it can be concluded that centrally administered leptin can improve hyperglycemia so as to bring STZ-D rats to euglycemia without altering serum insulin levels and that this effect is independent of peripheral action, or anorexic effects of leptin.

Recent studies have shown that peripheral administration of leptin manifests anti-diabetic effects not only in leptin-deficient *ob/ob* mice (11), but also in congenital lipodystrophic diabetic mice with severe insulin resistance (12, 13) and insulin-deficient STZ-D rats (14). The mechanism by which the anti-diabetic actions induced by leptin are regulated, especially whether it involves peripheral or central receptors, had not yet been elucidated completely. The present study demonstrates that chronic i3vt infusion of leptin reverses STZ-induced hyperglycemia completely. According to the observations of the STZ-LEPsc group, which was designed to rule out the possibility of leptin leakage into the systemic circulation, peripheral administration of a dose of leptin identical to the i3vt infusion did not reverse the hyperglycemia. These findings lead us to conclude that the main targets of leptin action through which euglycemia is restored in STZ-D rats reside in the brain, particularly in the hypothalamus.

The precise mechanism by which leptin restores insulin-deficient STZ-D rats to euglycemia through the CNS is unclear. It seems unlikely that leptin improves glucose metabolism by increasing insulin secretion, because i3vt leptin infusion left peripheral insulin concentration unaffected in the present study. Another possibility, that the reduction in food intake after leptin infusion may lead to improvement of glycemia in STZ-D rats, was evaluated by studying STZ-PF rats for which the food intake was identical to the STZ-LEP group. We found, however, that food restriction per se does not contribute to improvement of hyperglycemia in STZ-D rats. Indeed, previous findings have shown that food deprivation induces insulin resistance rather than insulin sensitivity in such rats (18, 25, 26).

An alternative, and perhaps most likely, possibility is that i3vt leptin infusion contributes to the regulation of key genes that encode glucose utilization and energy expenditure in peripheral tissues. In support of this hypothesis, STZ-D rats are impaired in the expression

TABLE 1. *The effects of chronic i3vt infusion of leptin or pair-feeding on expression of various genes in nondiabetic control rats*

	CIT-SAL	CIT-LEP	CIT-PF
BAT			
GLUT4	100.0 ± 9.1	143.3 ± 8.0^a	90.0 ± 9.2*
UCP1	100.0 ± 11.6	218.7 ± 20.4^a	74.9 ± 39.4†
UCP3	100.0 ± 9.3	103.8 ± 8.4	67.4 ± 10.5^{a,*}
Liver			
GLUT2	100.0 ± 6.7	34.6 ± 6.5^b	112.9 ± 9.3‡
G-6-Pase	100.0 ± 4.4	48.8 ± 7.7^c	102.5 ± 9.7‡
GK	100.0 ± 6.3	64.6 ± 2.2^b	13.4 ± 2.8^{c,‡}
PEPCK	100.0 ± 3.7	103.8 ± 7.3	105.6 ± 8.4
Muscle			
GLUT4	100.0 ± 1.2	106.1 ± 8.0	105.5 ± 5.4

^{a} = $P < 0.05$, ^{b} = $P < 0.001$, ^{c} = $P < 0.0001$ compared with the CIT-SAL group. * = $P < 0.05$, † = $P < 0.01$, ‡ = $P < 0.0001$ compared with the CIT-LEP group. Each value is a mean ± SE. n = 4 in each group. CIT-SAL: citrate buffer control group infused i3vt with saline. CIT-LEP: nondiabetic CIT group infused i3vt with leptin. CIT-PF: nondiabetic CIT controls pair-fed with the CIT-LEP group.

of genes mainly related to glucose metabolism and energy expenditure (24).

First, expression of genes in the liver that regulate glucose metabolism were examined, because glucose homeostasis had been found to be impaired in the liver of STZ-D rats (27, 28, 29). In the STZ-SAL group, expression of GLUT2 as a marker for glucose uptake and/or release from liver (24, 28) and G-6-Pase, an enzyme of gluconeogenesis, were up-regulated significantly whereas expression of GK mRNA, which encodes a major regulatory glycolytic enzyme, was reduced to less than half of that found in control animals. These results indicate that the amount of glucose produced in and released from the liver is accelerated in the insulin-deficient diabetic state. Indeed, a greater efflux of glucose from the liver to the systemic circulation occurs under insulin-deficient conditions (27, 28). Together with the increase in glucose efflux and gluconeogenesis, suppression of GK activity in the liver leads to acceleration of hyperglycemia as well. Central leptin infusion to the STZ-D rats restored GLUT2 and G-6-Pase mRNA levels completely and those of GK mRNA partially. GLUT2 and G-6-Pase mRNA expressions were affected slightly by pair-feeding, but much less than by leptin infusion. GK mRNA expression was not affected by pair-feeding. The recovery of gluconeogenic and glycolytic mRNA in liver is quite likely to be a major factor contributing to normalized glucose metabolism in the STZ-LEP group. In contrast, up-regulation of PEPCK expression in STZ-D rats was not reversed by i3vt leptin infusion, implying that gluconeogenesis (for which PEPCK is a crucial enzyme) may not be a major pathway for homeostasis of glucose metabolism enhanced by central leptin infusion in STZ-D rats.

Next, expression of GLUT4, an insulin-responsive glucose transporter, was examined in BAT and skeletal muscle, which together with white adipose tissue (WAT), are the primary sites of GLUT4 expression. WAT had clearly atrophied in size in STZ-SAL rats. WAT in STZ-LEP and STZ-PF rats was further atrophied compared with the STZ-SAL group. For that reason, we could not extract enough RNA for Northern blotting. Indeed, leptin reverses insulin resistance and diabetes even in fatless mice with congenital lipodystrophy (12, 13). Centrally infused leptin increased glucose uptake in BAT, heart, and skeletal muscle but not in WAT under nondiabetic conditions (8, 15, 16). WAT seems to be less significant for the anti-diabetic effects of leptin. In accordance with a previous report (30), expression of GLUT4 in STZ-D rats was down-regulated in BAT. Central leptin infusion restored the reduced expression of GLUT4 mRNA to control levels. Heart GLUT4 mRNA expression in STZ-D rats was down-regulated compared with the CIT-SAL group, and central leptin infusion restored the reduced expression of heart GLUT4 mRNA to control levels (unpublished observation). Another critical element of normalizing serum glucose concentration in diabetic rats may thus be attributable to the leptin-induced restoration of glucose transport. GLUT4 mRNA in skeletal muscle of STZ-D rats, however, was not affected by leptin infusion in the present study. This is inconsistent with previous reports that leptin increased glucose uptake into skeletal muscle under normoinsulinemic condition (8, 15, 16). There is no definitive explanation for this discrepancy, but a possible cause may be that insulin action cannot be expected in the present study (in which STZ-D rats were used) as opposed to the foregoing reports (in which intact rats were used). It would be unreasonable to assume that the transcriptional regulation of GLUT4 mRNA in skeletal muscle is not essential for leptin-induced modulation of glucose metabolism (31, 32). Intrinsic activity of GLUT4 translocation and/or the transporter recycling rate can alter glucose uptake without influencing actual expression of GLUT4 (33). There may be other potential biochemical or physiological mechanisms by which leptin can enhance glucose metabolism without altering GLUT4 expression in skeletal muscle directly.

Third, energy expenditure in the diabetic state was examined. Consistent with our previous study (34), STZ treatment in the present study down-regulated BAT UCP1 and UCP3 mRNA in STZ-D rats. Central leptin infusion restored their mRNA levels partially or completely. This restoration of UCP expression by leptin infusion may be at least in part another factor that enhances glucose uptake in STZ-D rats, because glucose uptake has been shown to be increased by chemical uncouplers, such as 2,4-dinitrophenol, in L6 and 3T3-L1 cells and by overexpression of UCP1 and UCP3 (35–37). In contrast to BAT-expressed UCP family members, as described here and in our previous report (34), changes in UCP3 mRNA in skeletal muscle did not contribute to the improvement of glucose metabolism by central leptin administration because leptin treatment decreased UCP3 mRNA expression in skeletal muscle.

Fourth, parameters that signify fatty acid utilization were examined. Expression of LPL, HSL, and CPT I mRNA is down-regulated in BAT of STZ-treated rats, which signifies a decrease in fatty acid uptake and β-oxidation. The fact that metabolic markers for fatty acid uptake and β-oxidation improved with leptin administration implies that glucose consumption was increased through enhanced UCP activity in BAT. In fact, utilization of glucose increases markedly during stimulated thermogenesis in association with translocation of glucose transporters to the plasma membrane (38, 39). Under insulin-deficient conditions such as fasting and insulin-dependent diabetes mellitus, skeletal muscle obtains energy by oxidizing fatty acids rather than glucose as its principal fuel source (40). Genes involved in fatty acid metabolism and β-oxidation in skeletal muscle were up-regulated after STZ treatment but returned to normal after leptin was centrally administered. Although skeletal muscle GLUT4 mRNA levels did not change in STZ-D rats, even upon subsequent treatment with leptin, markers of fatty acid utilization in skeletal muscle were up-regulated in the STZ-D rats and returned to normal when the rats were

treated centrally with leptin. Thus, it can be surmised that skeletal muscle switches its energy source from glucose to fatty acids and back to glucose, which implies that glucose uptake in muscle was first diminished and subsequently improved as the rat became diabetic, then returned to euglycemia.

To elucidate whether the effects of centrally infused leptin on genetic regulation in peripheral tissues is specific for insulin-deficiency or more generally applicable, we examined the effects of centrally administered leptin on control animals and its pair-fed group. GLUT4 and UCP1 in BAT and GLUT2 and G-6-Pase in liver were affected similarly by leptin administration regardless of insulin status. Changes in BAT UCP3 and liver GK mRNA expression in nondiabetic control rats treated with leptin seemed to be different from those in STZ-LEP rats. However, the effects of leptin on expression of these genes in nondiabetic rats were consistent with those on STZ-D animals vs. the pair-fed group. In other words, although pair-feeding down-regulated BAT UCP3 and liver GK mRNA expression in nondiabetic control rats, central leptin infusion prevented the further reduction in gene expression by pair-feeding. These findings imply that leptin exerts its rescue by acting on gene expression in general and not exclusively in insulin-deficient animals. Regulation of gene expression in the liver, as shown in the present study, is both consistent (29) and inconsistent (9, 10, 17) with previous studies. There has been no evidence to suggest or explain such a discrepancy. One possibility may be differences in experimental protocols, such as the route of leptin administration (9, 10), experimental period (17), or pancreatic or insulin clamps used in the previous studies (9, 10, 17).

It is useful to note the difference in body weight between STZ-LEP and STZ-PF rats. Leptin-induced feeding suppression was expected to induce additional weight loss in STZ-LEP rats (41). Instead, food restriction matched with STZ-LEP rats caused obvious weight reduction in STZ-PF rats compared with STZ-SAL rats, but body weight reduction in the STZ-LEP group was equivalent to that in the STZ-SAL group. Additive effects of leptin on weight reduction could not be observed in STZ-LEP rats. These findings may indicate that leptin-induced normalization of diabetes prevents further weight reduction. These results raise the question of why the STZ-LEP group lost and CIT-SAL controls gained weight despite the lack of any difference in food intake or serum glucose concentration between the groups. A similar finding is noted in a previous report (14). A possible explanation is that the glucose-lowering mechanism of leptin is different from that of insulin. When insulin is injected into STZ-D rats, there is an immediate regeneration of atrophied WAT along with a drop in serum glucose. The regenerated WAT will result in the normalization of serum leptin, which will ameliorate the hyperphagia of STZ-D rats (34, 42, 43). However, as mentioned above, central infusion of leptin ameliorates glucose concentration in STZ-D rats without hypertrophy of WAT; on the contrary, WAT typically atrophies. Since serum insulin is preserved in the CIT-SAL group, body WAT steadily accumulates. This is not true in the insulin-depleted STZ-LEP group. The difference in WAT accumulation translates into the difference in body weight.

Finally, there is the question of how the signals that regulate glucose utilization and energy expenditure in peripheral tissues are transmitted from the CNS. Involvement of efferent sympathetic nerves is the most likely explanation for transduction of the leptin message from the CNS to peripheral tissues. The sympathetic nervous system has been found to be involved in the modulation of glucose uptake and utilization in peripheral tissues independent of insulin action (44, 45). The accelerated glucose uptake in BAT and skeletal muscle in response to central leptin infusion is attenuated by sympathetic denervation (8, 15, 16). Direct measurement of sympathetic nerve activity shows that leptin infusion increases the activity of nerves going to BAT and hind limbs (46, 47). Another possible explanation for the modulation of glucose metabolism is a pathway through a neuroendocrine system. Leptin administration is found to significantly increase the rate of conversion of T4 to T3 and the concentration of thyroid hormones (8, 14), which in turn elevates the rate of glucose transport (48). There may well be other unidentified glucose-lowering signals released by leptin into the CNS.

In summary, we have demonstrated that chronic i3vt infusion of leptin is effective for improving hyperglycemia in STZ-D independent of its effects on food intake and systemic insulin concentration. Serum glucose can be regulated by the CNS upon administration of leptin at such small doses that it has no significant peripheral effect. Leptin may contribute to the improvement of abnormal levels in gene expression of GLUT2, G-6-Pase, and GK in liver, GLUT4 in BAT, and UCP family members in BAT that presumably is mediated by the sympathetic nervous and/or neuroendocrine systems. This discovery may lead to the development of a new class of anti-diabetic drugs that act on the CNS. We now rely on insulin release enhancers (sulfonylureas), insulin sensitivity enhancers (thiazolidinedione), and insulin itself to clinically control blood glucose. Additional study of leptin and its downstream regulators may lead to the development of drugs that may free us from our dependence on insulin-related remedies. In light of the prevalence of obesity and diabetes in the developed world today, the importance of continued investigation in this field cannot be overstated. FJ

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