Acute Central Infusion of Leptin Modulates Fatty Acid Mobilization by Affecting Lipolysis and mRNA Expression for Uncoupling Proteins

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Chronic administration of leptin has been shown to reduce adiposity through energy intake and expenditure. The present study aims to examine how acute central infusion of leptin regulates peripheral lipid metabolism, as assessed by markers indicative of their mobilization and utilization. A bolus infusion of 1 μg/rat leptin into the third cerebroventricle increased the expression of mRNA for hormone-sensitive lipase (HSL), an indicator of lipolysis, in white adipose tissue (WAT). This was accompanied by elevation of plasma levels of glycerol, but not of free fatty acids, as compared to the saline control ($P < 0.03$). The same treatment with leptin decreased plasma insulin levels but did not affect the plasma glucose level ($P < 0.05$ for insulin). Among the major regulators of the transportation or utilization of energy substrates, leptin treatment increased expression of mRNA for uncoupling protein 1 (UCP1) in brown adipose tissue (BAT), UCP2 in WAT, and UCP3 in quadriceps skeletal muscle, but not those for fatty acid–binding protein in WAT, carnitine phosphate transferase-1, a marker for β oxidation of fatty acids in muscle, nor glucose transporter 4 in WAT and muscle ($P < 0.01$ for HSL, $P < 0.05$ for UCP1, and $P < 0.005$ for UCP2 and UCP3). These results indicate that, even in a single bolus, leptin may regulate the mobilization and/or utilization of energy substrates such as fatty acids by affecting lipolytic activity in WAT and by increasing the expression of UCPs in BAT, WAT, and muscle. Exp Biol Med 230:200–206, 2005

Key words: leptin; uncoupling protein (UCP); energy expenditure; lipolysis; hypothalamus

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Introduction

Leptin, an *ob* gene product that is secreted from adipose tissue, plays an essential role in the regulation of feeding behavior and energy metabolism (1). A growing body of evidence indicates that both a decrease in food intake and an increase in energy expenditure following central as well as peripheral administration of leptin can induce loss of body fat mass (2, 3). Indeed, central infusion of leptin has been shown to elevate energy expenditure, as assessed by both O_2 consumption and mRNA expression for uncoupling protein 1 (UCP1) in brown adipose tissue (BAT), this being another indicator for peripheral energy expenditure (4–8).

BAT UCP1 plays a major role in cold- or diet-induced thermogenesis in rodents. This thermogenic action of UCP1 promotes energy expenditure as a result of the utilization of energy substrates such as glucose and fatty acid (9). Newly identified UCP homologs UCP2 and UCP3 have also received a lot of attention in relation to their control of energy metabolism, because these homologs are detectable not only in small mammals but also in adult humans (10, 11). However, apart from BAT UCP1, the physiological role of the remaining UCP family has remained unclear. Recently it has been demonstrated that UCP3 may contribute to the exportation of fatty acids from the mitochondrial matrix rather than to the regulation of energy expenditure through thermogenesis (12). This finding also indicates that, in the assessment of UCP function, it is important to analyze not only thermogenesis and/or energy expenditure but also the utilization and/or mobilization of energy substrates. It is well known that these UCP expressions are under the control of humoral factors, as well as neural inputs from the sympathetic nervous system. For example, leptin may regulate UCP function directly or indirectly through the hypothalamic-sympathetic nervous system axis. Chronic central leptin infusion is known to upregulate UCP1–UCP3 in BAT, UCP2 in white adipose tissue (WAT), and UCP3 in skeletal muscle more predom-

inantly than in their pair-fed controls (13). From these results, it is highly probable that the action of leptin on UCP1–UCP3, in addition to its effect on energy balance, may contribute to the central regulation of energy utilization and/or its mobilization.

There have been significant data to indicate that leptin is involved in the regulation of peripheral lipid and glucose metabolism (14–16). An *in vitro* study of leptin showed an increase of lipolytic activity in adipocytes, as assessed by glycerol release into the incubation medium (17). An *in vivo* study has also demonstrated that leptin administration (18) or transfer of the leptin gene into the muscle (7) accelerated lipolytic action in adipose tissues. In the liver, peripheral treatment with leptin prevented accumulation of lipids by the acceleration of fatty acid oxidation (19). In addition, leptin has been shown to stimulate fatty acid oxidation in muscle by activating 5′-AMP activated protein kinase (AMPK) through direct peripheral action, as well as by centrally mediated action (20). Thus, it could be concluded that leptin regulates peripheral lipid metabolism at each step by lipolysis, fatty acid oxidation, and energy expenditure. However, it remains unclear whether these effects are centrally or peripherally mediated.

Chronic treatment with leptin induces loss of body weight and fat mass, which may secondarily affect the body's metabolism and hormone secretion. These alterations in body composition and humoral factors *per se* may have the capability to induce changes in a variety of metabolic parameters, such as UCPs. Thus, to assess centrally mediated direct leptin action on peripheral energy metabolism, it is particularly important to exclude the involvement of factors such as changes in food intake, adiposity, and humoral factors induced by chronic leptin treatment.

The present study aims to clarify how acute central administration of leptin regulates peripheral lipid metabolism, using markers that indicate utilization and/or mobilization of energy substrates.

Materials and Methods

Animals. Mature male Wistar King A rats weighing 300–360 g at 11 to 12 weeks of age were used. They were housed in a room that was illuminated daily from 0700 hrs to 1900 hrs (a 12:12-hr light:dark cycle) and maintained at 21°C ± 1°C with humidity at 55% ± 5%. Rats were allowed free access to pellet rodent chow (CE-2; CLEA Japan Ltd, Tokyo, Japan) and tap water. All studies were conducted in accordance with the Oita Medical University Guidelines, based on the National Institutes of Health *Guide for the Care and Use of Laboratory Animals.*

Reagents. Recombinant murine leptin (Amgen, Thousand Oaks, CA) was dissolved in saline to a concentration of 0.1 μg/μl. Each solution was freshly prepared on the day of its administration. The pH of each solution was adjusted to 6.4–7.2.

Third Cerebroventricle (i3vt) Infusion. Under anesthesia with intraperitoneal sodium pentobarbital injection (45 mg/kg), the rats were fixed in a stereotaxic apparatus (Narishige Co., Tokyo, Japan) so that a stainless-steel guide cannula (23 gauge) could be chronically implanted into the i3vt region at least 1 week before infusion of the test solution. A stainless-steel wire stylet (29 gauge) was left in the guide cannula to prevent leakage of cerebrospinal fluid or obstruction of the cannula. All rats that had undergone surgery of the i3vt or the jugular vein (described below) were allowed to recover for 1 week and were handled for 5 min daily before each experiment to equilibrate their arousal levels. Details of the surgical procedures have been described elsewhere (21).

By 1 day before the infusion study, the rats had recovered, at least, to the food intake level and body weight observed before the surgical procedures were performed. Matched on the basis of body weight on the testing day, 16 rats were equally divided into two groups, one treated with leptin ($n = 8$) and the other serving as the saline control ($n = 8$) solution. On the testing day, the rats fasted for 3 hrs. Leptin at a dose of 1 μg/rat was infused to the i3vt for 10 mins with an infusion rate of 1.0 μl/min from 1000 hrs to 1030 hrs, while rats were unanesthetized and unrestrained. The procedures for saline infusion in the control group were the same as those for the leptin-infusion group. Each rat was sacrificed 3 hrs after the infusion. After sacrifice, BAT, the epididymal fat tissue (WAT), and the quadriceps skeletal muscle were extirpated, immediately frozen in liquid nitrogen, and stored at −80°C until RNA extraction.

Blood Sampling and its Assay. Blood samples were collected from each rat through a chronically indwelling silicone catheter implanted in the right external jugular vein, with its end at a point immediately outside of the right atrium. Details of the sampling procedures have been described elsewhere (22). A blood sample was collected at 3 hrs after the infusion and quickly separated into plasma. These plasma samples were immediately frozen at −20°C until humoral factors were measured. Plasma insulin concentrations were quantified by a double antibody radioimmunoassay using [^{125}I]labeled rat insulin (Amersham, Bucks, UK). Plasma glucose concentration was measured by the glucose electrode method (Antsense; Mile-Sankyo, Tokyo, Japan). Free fatty acid (FFA) concentration was measured by an enzymatic colorimetric technique with a reagent kit (Wako Pure Chemicals USA, Inc., Richmond, VA). Glycerol concentration was measured by commercial kit (F-kit glycerol; Boehringer Mannheim K.K., Tokyo, Japan)

RNA Extraction and Northern Blot Analysis. Rats were sacrificed 3 hrs after i3vt infusion of leptin; following this, the intrascapular brown adipose tissue, the epididymal adipose tissue, and quadriceps skeletal muscle were isolated for Northern blot analysis. Total cellular RNA was prepared from BAT, WAT, and skeletal muscle using Trizol (Gibco, Grand Island, NY), according to the

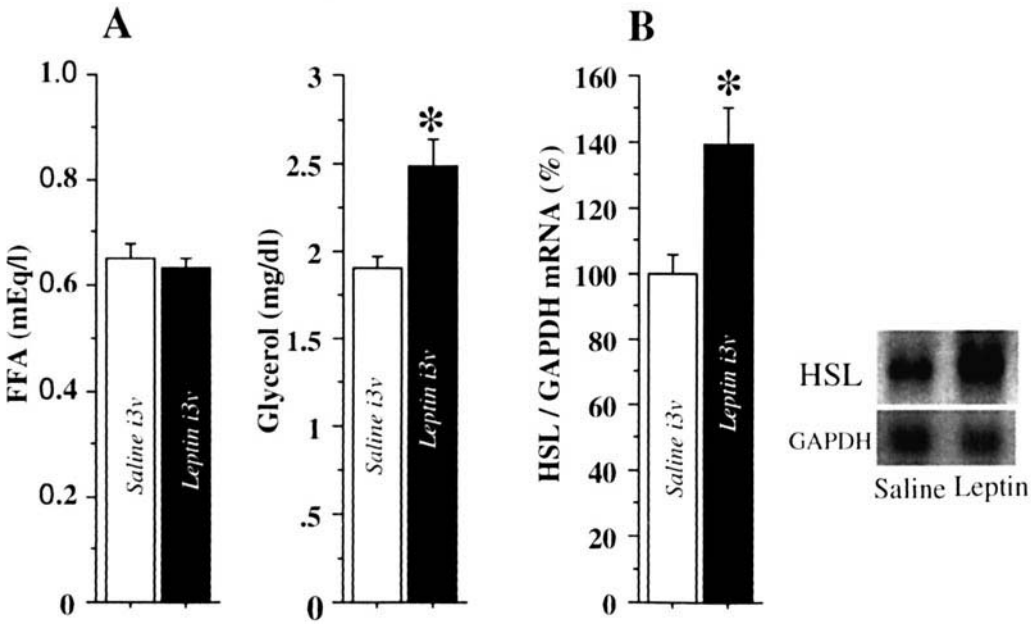

Figure 1. Changes in serum free fatty acid (FFA) and glycerol concentrations (A) and hormone-sensitive lipase (HSL) mRNA expression in epididymal white adipose tissue (B) 3 hrs after 1 μg/rat leptin or saline infusion into the third cerebroventricle (i3vt). In this and succeeding figures, i3vt represents infusion into the third cerebroventricle. Each value (n = 8, mean ± SEM.) was measured 3 hrs after administration of leptin (1 μg/rat) i3vt. HSL values are expressed in arbitrary units, with the values for HSL mRNA levels in animals that received saline arbitrarily set at 100, and the SEM. adjusted proportionally. * $P < 0.05$ versus saline control group.

manufacturer's protocol. Total RNAs (20 μg/lane) were electrophoresed on 1.2% formaldehyde-agarose gel. The separated RNAs were transferred onto a Biodyne B membrane (Pall Canada, Toronto, Canada) in 20×SSC by capillary blotting and immobilized by exposure to ultraviolet light (0.8 J). cDNA probes for rat UCP1, UCP2, UCP3, hormone-sensitive lipase (HSL), glucose transporter 4 (GLUT4), adipocyte fatty acid–binding protein (aP2), and carnitine palmitoyltransferase-1 (CPT1) were prepared by reverse transcription–polymerase chain reaction by use of the following primers: UCP1 sense, 5′-AGTGCCACTGTTGTCTTCAG-3′; UCP1 antisense, 5′-TTCTCCAAGTCGCCTATGTG-3′; UCP2 sense, 5′-CATCTTCTGGGAGGTAGC-3′; UCP2 antisense, 5′-AAGACAGGGCAGGAATGG-3′; UCP3 sense, 5-GTTACCTTTCCACTGGACAC-3′; UCP3 antisense, 5′-CCGTTTCAGCTGCTCATAGG-3′; HSL sense, 5′-ACATGGCCTTCTTCTCAAGC-3′; HSL antisense, 5′-TCATGGGATTTGGAGGTCTG-3′; GLUT4 sense, 5-TCTGGCTATCACAGTACTCC-3′; GLUT4 antisense, 5-TCTGTACTGGGTTTCACCTC-3′; ap2 sense, 5′-TGTGATGCCTTTGTGGGGAC-3′; ap2 antisense, 5′-TGCTCTTTCATAAACTCTTG-3′; CPT1 sense, 5′-ATTTTGGCGACAGACTCAGG-3′; and CPT1 antisense, 5′-AGGGGCAGGAATCAAACAAG-3′. Identification of the appropriately sized product was carried out by mapping with multiple restriction endonucleases and sequencing. cDNA probe was labeled by random prime labeling kit (Amersham Biosciences Corp., Piscataway, NJ) with [α-^{32}P] dCTP. Prehybridization and hybridization were carried out according to the manufacturer's protocol. The blots were hybridized using the ^{32}P-labeled cDNA fragment at 42°C for 20 hrs in the presence of salmon sperm DNA. Membranes were washed under high-stringency conditions. After washing the membranes, the hybridization signals were analyzed with a BIO-image analyzer BAS 2000 (Fuji Film Institution, Tokyo, Japan). The membranes were stripped by exposure to boiling 0.1% sodium dodecyl sulfate and rehybridized with a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA probe (Wako, Osaka, Japan), which was used to quantify the amounts of RNA species on the blots in each sample. Band intensities obtained by Northern blots were normalized to the signal obtained after stripping and reprobing the same membranes with ^{32}P-labeled GAPDH cDNA probe. We quantified the amount of both of the signals, target gene and GAPDH, divided the signal of target mRNA by that of GAPDH, and then converted the results to a percentage. The results were expressed as mean ± SEM.

Statistical Analysis. All the data are expressed as the mean ± SEM. The statistical significance of the differences was assessed by unpaired t test. Values of $P < 0.05$ were accepted as being statistically significant.

Results

Effect of Central Infusion of Leptin on Lipid Metabolism. Figure 1 shows changes in plasma concentrations of glycerol, FFA, and mRNA expression of HSL in the WAT 3 hrs after i3vt leptin infusion. The leptin infusion increased serum glycerol concentration by 31% (each group, n = 8, $P < 0.03$), relative to saline control infusion (Fig.

Table 1. Plasma Glucose (PG) and Insulin (IRI) Concentration in Rats Infused with Leptin or Saline Acute Intracerebroventricularly (i3vt)[a]

	Saline (i3vt)	Leptin (i3vt)
PG (mg/dl)	124.5 ± 4.2	128.0 ± 4.4
IRI (ng/ml)	15.0 ± 1.0	9.7 ± 1.8*

[a] Data are mean ± SEM. Statistical significance: each group, $n = 8$.
* $P < 0.05$ versus the saline (i3vt) group.

1A), but it did not affect serum FFA. Leptin treatment increased mRNA expression of WAT HSL mRNA by 39%, as compared to saline infusion (each group, $n = 8$, $P < 0.01$) (Fig. 1B). However, these short-term effects of leptin on WAT lipid metabolism did not affect body weight and adiposity (data not shown). The effects of leptin concern the parameters relating to transportation and utilization of FFA. Leptin did not affect WAT aP2 or muscle CPT1 gene expression (data not shown).

Effects of Central Infusion of Leptin on Glucose Metabolism. Changes in serum glucose and insulin concentrations after leptin infusion are shown in Table 1. Leptin decreased the serum insulin to 64.7% of that of the saline control (each group, $n = 8$, $P < 0.05$), but serum glucose was unaffected (Table 1). Leptin, however, did not affect WAT or muscle GLUT4 mRNA (data not shown).

Leptin-Induced Upregulation of the UCP Family. Figure 2 shows changes in mRNA expression of the UCP family 3 hrs after i3vt leptin infusion. Leptin upregulated BAT UCP1 mRNA by 28% (each group, $n = 8$, $P < 0.05$), but not BAT UCP2 or UCP3 mRNA, relative to the saline infusion (Fig. 2A and D). Leptin upregulated WAT UCP2 mRNA by 37% (each group, $n = 8$, $P < 0.005$), but not WAT UCP3 mRNA (Fig. 2B and E). Skeletal muscle UCP3 mRNA was upregulated by 35% (each group, $n = 8$, $P < 0.005$) (Fig. 2C and F).

Discussion

The present study demonstrated that acute i3vt infusion of leptin accelerates lipolysis in WAT and yields upregulation of the UCP family in peripheral tissues. The triglycerides in adipose tissue are metabolized into FFA and glycerol in response to lipolytic messengers, such as catecholamine, glucagon, etc. Adipocyte-derived glycerol is transferred into the circulation without reuptake into adipose tissue. A proportion of FFA, however, is stored in adipose tissue *per se*, and the remainder is transported into muscle and the liver to be used as an energy substrate. From a metabolic point of view, the plasma concentration of glycerol is thus a more sensitive measure of lipolytic activity than that of FFA (17). Reflecting the different metabolic fates of glycerol and FFA, i3vt leptin infusion in the present study increased serum glycerol concentration, but not serum FFA. In addition to a change in glycerol level, the present study demonstrated that central leptin treatment increased HSL mRNA expression. HSL is presumed to be a key enzyme for the lipolytic process. However, it was recently demonstrated that a lipolytic agent such as isoproterenol could stimulate lipolytic activity, even in HSL-deficient mice, although its effect was lesser than that noted in wild-type mice, indicating mediation by an HSL-independent lipolytic factor (23). Although the involvement of another lipolytic mechanism was unclear in the present study, it can be concluded that upregulation of HSL mRNA in response to central leptin treatment may explain the accelerating effect of leptin on lipolysis.

Insulin inhibits lipolysis through HSL inactivation. An antagonistic leptin effect on insulin-induced lipogenesis has been shown in isolated adipocyte (17). Although the effects of peripheral leptin on insulin secretion, insulin sensitivity, and glucose metabolism have been investigated (16), it remains unclear whether centrally administered leptin can modulate the action of peripheral insulin on adipocytes. i3vt leptin infusion lowered serum insulin concentration in the present study. One possible explanation is that leptin-induced activation of sympathetic nerves may inhibit insulin secretion from the pancreas. A previous study supports this possibility, as plasma insulin concentration was shown to be markedly decreased by sympathetic activation (24, 25). Serum glucose concentration in the present study, however, was left unaffected, regardless of the induction of hypoinsulinemia. From these findings, it can be postulated that central leptin treatment may improve insulin sensitivity and/or glucose utilization. Nevertheless, i3vt leptin infusion in the present study failed to upregulate GLUT4 mRNA in either the WAT or skeletal muscle. This result does not necessarily exclude the possibility that central administration of leptin may affect glucose uptake. Indeed, systemic administration of leptin decreased plasma glucose concentration without a change in GLUT4 content (26). Recently it has been demonstrated that central leptin administration activates AMPK (20). Activation of this enzyme has been shown to promote fatty acid oxidation and insulin-independent glucose transportation (20). This unique interaction between leptin and AMPK may explain, to some extent, the apparent leptin-induced increase in glucose uptake indicated by the present study. Taken together, it can be said that lipolytic action following central leptin infusion is induced mainly by sympathetic activation, but not by hypoinsulinemia, although partial involvement of hypoinsulinemia cannot be completely excluded.

Acute central administration of leptin in the present study upregulated gene expression for UCPs in adipose tissue and skeletal muscle in a tissue-specific manner. Leptin upregulated UCP1 mRNA, but not that of UCP2 or UCP3 in BAT. Both thermogenesis and energy expenditure in BAT are mediated by UCP1, under the control of the efferent sympathetic nervous system (27). The present result is consistent with those of numerous previous studies that indicate the essential role of central leptin action in the

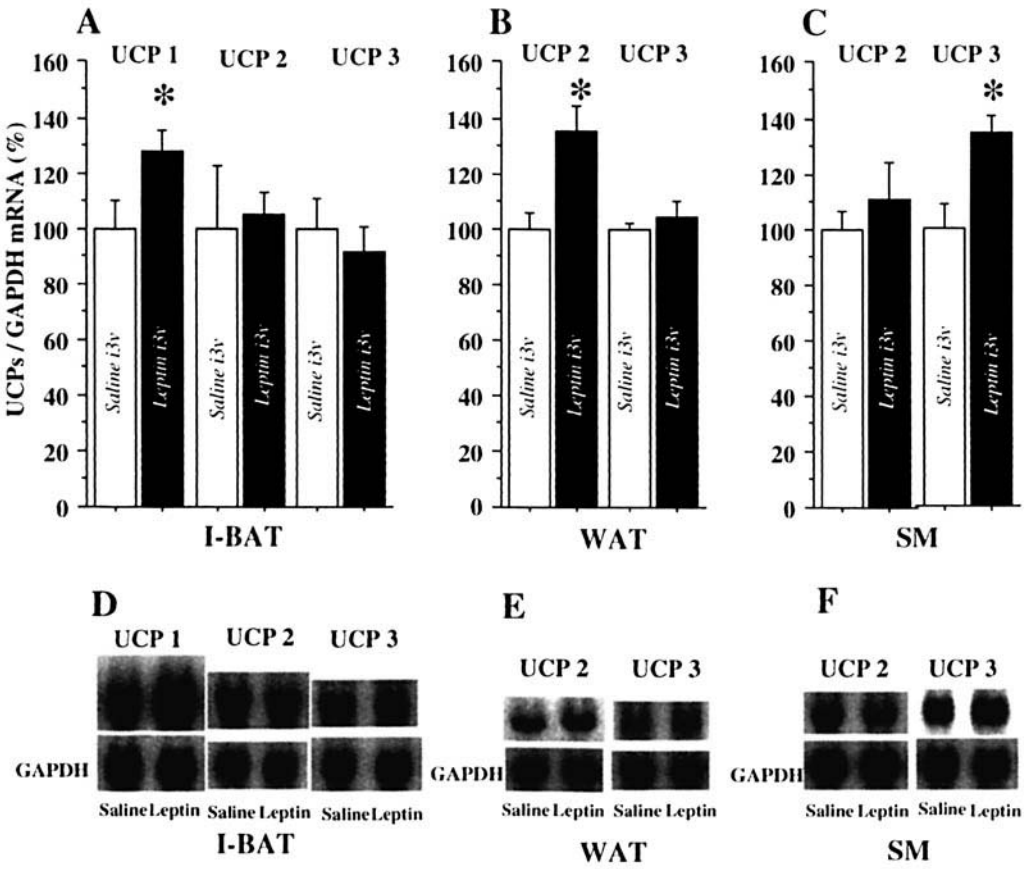

Figure 2. Gene expressions of uncoupling protein (UCP) family in the intrascapular brown adipose tissue (I-BAT) (A, D), epididymal white adipose tissue (WAT) (B, E), and the skeletal muscle (SM) (C, F) 3 hrs after infusion into the third cerebroventricle (i3vt), 1 μg/rat leptin or saline infusion. Values (mean ± SEM) are expressed as % difference in arbitrary units between leptin and saline i3vt infusion (each $n = 8$). * $P < 0.05$ versus saline control group.

regulation of energy balance (by affecting food intake) and of energy expenditure by upregulation of BAT UCP1 (13, 28). Furthermore, this centrally mediated leptin action may contribute to an increase in energy utilization through the BAT thermogenic process using energy substrates such as glucose and fatty acids.

In the present study, central leptin infusion increased WAT UCP2 mRNA and muscle UCP3 mRNA expression. This result is also consistent with that of a previous study using chronic leptin administration (13). Gene expression of both WAT UCP2 and muscle UCP3 is known to be upregulated by administration of a β_3 adrenoceptor agonist (29, 30). Thus, in a similar way to that noted with BAT UCP1, WAT UCP2 and muscle UCP3 are influenced by the sympathetic nervous system, as well as by central leptin action. However, the functional meaning of this regulatory system for energy metabolism, especially the role of UCP2, remains unclear. Recently it has been proposed that the primary physiological role of UCP3 may be the mitochondrial handling of fatty acids rather than the regulation of energy expenditure through thermogenesis (12). Exportation of fatty acids from the mitochondrial matrix by UCP3 may offer protection from the accumulation of fatty acids in mitochondria and may help to maintain muscular fat oxidative capacity. Thus, leptin-induced upregulation of muscle UCP3 may also contribute to central regulation of fatty acid mobilization and utilization. As another candidate for the role of modulator of UCP3, it is necessary to discuss the function of FFA, *per se*, released from adipose tissue. Among peripheral factors that regulate UCPs such as leptin, glucocorticoid, thyroid hormone, and tumor necrosis factor (TNF)-α, FFA has been shown to upregulate skeletal muscle UCP3 expression (31–36). In the present study, the plasma FFA level did not change after leptin treatment. However, FFA possibly has the ability to affect UCP3 in the present study since it has been released from adipose tissue, similar to the glycerol that increased in the blood level.

Our results show that central leptin treatment accelerates lipolysis and upregulates UCP expression. This finding led us to hypothesize that central leptin administration might not simply regulate each peripheral mechanism related to lipid metabolism individually; rather, it seemed to contribute to coordination between each process, from energy supply to its utilization. This indicated that it might be interesting to investigate whether central leptin treatment could regulate other steps of fatty acid mobilization and/or utilization. To assess this, we analyzed gene expression of aP2 in WAT, a marker of fatty acid

transportation, and CPT1 in skeletal muscle, a marker of fatty acid oxidation. The results showed that there were no significant changes in the expression of either parameter. However, the possibility that fatty acid oxidation may be modulated by centrally mediated leptin action still remains. First, muscle UCP3 upregulated by leptin may affect it (37–39). Second, as described above, central administration of leptin has been shown to activate AMPK, which can stimulate fatty acid oxidation (20). It is likely that measurement of indicators other than CPT1 mRNA may be necessary to analyze the detailed mechanism of this central action. Thus, the present study indicates that acute leptin treatment may affect mobilization and/or utilization of lipid. In the chronic stage, further metabolic change may develop from this initial response.

Finally, we must discuss the central mechanism of leptin action in the regulation of peripheral lipid metabolism. A possible pathway in the central regulation of BAT UCP1 may be mediated by pro-opiomelanocortin (POMC) neurons in the arcuate nucleus, one of the major targets for leptin action in the hypothalamus. Direct neuronal projection of POMC neurons, as well as expression of melanocortin 4 receptors, has been identified in sympathetic preganglionic neurons of the intermediolateral cell column of the spinal cord (IML) (40). Indeed, our previous study demonstrated that central infusion of α-melanocyte–stimulating hormone, a POMC-derived peptide, increased BAT sympathetic nerve activity (41–43). As a second possibility, direct leptin effects on the paraventricular nucleus (PVN) can be considered, since localization of the leptin receptor is identified in this region. The PVN also directly projects to the IML (44). Indeed, stimulation of this nucleus activates BAT sympathetic nerves (45).

In summary, acute i3vt infusion of leptin may modulate mobilization and/or utilization of lipids through the acceleration of lipolysis in the WAT and through upregulation of BAT UCP1, WAT UCP2, and muscle UCP3 mRNA in rats.

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1. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature 372:425–432, 1994.
2. Campfield LA, Smith FJ, Guisez Y, Devos R, Burn P. Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. Science 269:546–549, 1995.
3. Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T, Collins F. Effects of the obese gene product on body weight regulation in *ob/ob* mice. Science 269:540–543, 1995.
4. Hwa JJ, Ghibaudi L, Compton D, Fawzi AB, Strader CD. Intracerebroventricular injection of leptin increases thermogenesis and mobilizes fat metabolism in *ob/ob* mice. Horm Metab Res 28:659–663, 1996.
5. Scarpace PJ, Matheny M, Pollock BH, Tumer N. Leptin increases uncoupling protein expression and energy expenditure. Am J Physiol 273:E226–E230, 1997.
6. Mistry AM, Swick AG, Romsos DR. Leptin rapidly lowers food intake and elevates metabolic rates in lean and *ob/ob* mice. J Nutr 127:2065–2072, 1997.
7. Marti A, Novo FJ, Martinez-Anso E, Zaratiegui M, Aguado M, Martinez JA. Leptin gene transfer into muscle increases lipolysis and oxygen consumption in white fat tissue in *ob/ob* mice. Biochem Biophys Res Commun 246:859–862, 1998.
8. Mistry AM, Swick A, Romsos DR. Leptin alters metabolic rates before acquisition of its anorectic effect in developing neonatal mice. Am J Physiol 277:R742–R747, 1999.
9. Nicholls DG, Locke RM. Thermogenic mechanisms in brown fat. Physiol Rev 64:1–64, 1984.
10. Fleury C, Neverova M, Collins S, Raimbault S, Champigny O, Levi Meyrueis C, Bouillaud F, Seldin MF, Surwit RS, Ricquier D, Warden CH. Uncoupling protein-2: a novel gene linked to obesity and hyperinsulinemia. Nat Genet 15:269–272, 1997.
11. Boss O, Samec S, Paoloni-Giacobino A, Rossier C, Dulloo A, Seydoux J, Muzzin P, Giacobino JP. Uncoupling protein-3: a new member of the mitochondrial carrier family with tissue-specific expression. FEBS Lett 408:39–42, 1997.
12. Rousset S, Alves-Guerra MC, Mozo J, Miroux B, Cassard-Doulcier AM, Bouillaud F, Ricquier D. The biology of mitochondrial uncoupling proteins. Diabetes 53:S130–S135, 2004.
13. Cusin I, Zakrzewska KE, Boss O, Muzzin P, Giacobino JP, Ricquier D, Jeanrenaud B, Rohner-Jeanrenaud F. Chronic central leptin infusion enhances insulin-stimulated glucose metabolism and favors the expression of uncoupling proteins. Diabetes 47:1014–1019, 1998.
14. Siegrist-Kaiser CA, Pauli V, Juge-Aubry CE, Boss O, Pernin A, Chin WW, Cusin I, Rohner-Jeanrenaud F, Burger AG, Zapf J, Meier CA. Direct effects of leptin on brown and white adipose tissue. J Clin Invest 100:2858–2864, 1997.
15. Fruhbeck G, Aguado M, Martinez JA. *In vitro* lipolytic effect of leptin on mouse adipocytes: evidence for a possible autocrine/paracrine role of leptin. Biochem Biophys Res Commun 240:590–594, 1997.
16. Kamohara S, Burcelin R, Halaas JL, Friedman JM, Charron MJ. Acute stimulation of glucose metabolism in mice by leptin treatment. Nature 389:374–377, 1997.
17. Muller G, Ertl J, Gerl M, Preibisch G. Leptin impairs metabolic actions of insulin in isolated rat adipocytes. J Biol Chem 272:10585–10593, 1997.
18. Fruhbeck G, Aguado M, Gomez-Ambrosi J, Martinez JA. Lipolytic effect of *in vivo* leptin administration on adipocytes of lean and *ob/ob* mice, but not *db/db* mice. Biochem Biophys Res Commun 250:99–102, 1998.
19. Shimabukuro M, Koyama K, Chen G, Wang MY, Trieu F, Lee Y, Newgard CB, Unger RH. Direct antidiabetic effect of leptin through triglyceride depletion of tissues. Proc Natl Acad Sci U S A 94:4637–4641, 1997.
20. Minokoshi Y, Kim YB, Peroni OD, Fryer LG, Muller C, Carling D, Kahn BB. Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. Nature 415:339–343, 2002.
21. Sakata T, Tsutsui K, Fukushima M, Arase K, Kita H, Oomura Y, Ohki K, Nicolaidis S. Feeding and hyperglycemia induced by 1,5-anhydroglucitol in the rat. Physiol Behav 27:401–405, 1981.
22. Fukushima M, Tsutsui K, Kodama J, Sakata T, Goto M, Teranishi T. New inexpensive device for estimating dry food intake in small animals. Physiol Behav 22:1025–1028, 1979.
23. Haemmerle G, Zimmermann R, Hayn M, Theussl C, Waeg G, Wagner E, Sattler W, Magin TM, Wagner EF, Zechner R. Hormone-sensitive lipase deficiency in mice causes diglyceride accumulation in adipose tissue, muscle, and testis. J Biol Chem 277:4806–4815, 2002.
24. Bloom SR, Edwards AV. The release of pancreatic glucagon and inhibition of insulin in response to stimulation of the sympathetic innervation. J Physiol 253:157–173, 1975.
25. Muzumdar R, Ma X, Yang X, Atzmon G, Bernstein J, Karkanias G,

Barzilai N. Physiologic effect of leptin on insulin secretion is mediated mainly through central mechanisms. FASEB J 17:1130–1132, 2003.

26. Sivitz WI, Walsh SA, Morgan DA, Thomas MJ, Haynes WG. Effects of leptin on insulin sensitivity in normal rats. Endocrinology 138:3395–3401, 1997.
27. Li G, Klein RL, Matheny M, King MA, Meyer EM, Scarpace PJ. Induction of uncoupling protein 1 by central interleukin-6 gene delivery is dependent on sympathetic innervation of brown adipose tissue and underlies one mechanism of body weight reduction in rats. Neuroscience 115:879–889, 2002.
28. Satoh N, Ogawa Y, Katsuura G, Numata Y, Masuzaki H, Yoshimasa Y, Nakao K. Satiety effect and sympathetic activation of leptin are mediated by hypothalamic melanocortin system. Neurosci Lett 249:107–110, 1998.
29. Emilsson V, Summers RJ, Hamilton S, Liu YL, Cawthorne MA. The effects of the beta3-adrenoceptor agonist BRL 35135 on UCP isoform mRNA expression. Biochem Biophys Res Commun 252:450–454, 1998.
30. Nakamura Y, Nagase I, Asano A, Sasaki N, Yoshida T, Umekawa T, Sakane N, Saito M. Beta 3-adrenergic agonist up-regulates uncoupling proteins 2 and 3 in skeletal muscle of the mouse. J Vet Med Sci 63:309–314, 2001.
31. Gong DW, He Y, Karas M, Reitman M. Uncoupling protein-3 is a mediator of thermogenesis regulated by thyroid hormone, beta3-adrenergic agonists, and leptin. J Biol Chem 272:24129–24132, 1997.
32. Ricquier D, Miroux B, Larose M, Cassard-Doulcier AM, Bouillaud F. Endocrine regulation of uncoupling proteins and energy expenditure. Int J Obes Relat Metab Disord 24(Suppl 2):S86–S88, 2000.
33. Masaki T, Yoshimatsu H, Sakata T. Expression of rat uncoupling protein family mRNA levels by chronic treatment with thyroid hormone. Int J Obes Relat Metab Disord 24(Suppl 2):S162–S164, 2000.
34. Masaki T, Yoshimatsu H, Chiba S, Hidaka S, Tajima D, Kakuma T, Kurokawa M, Sakata T. Tumor necrosis factor–alpha regulates *in vivo* expression of the rat UCP family differentially. Biochim Biophys Acta 1436:585–592, 1999.
35. Dulloo AG, Samec S, Seydoux J. Uncoupling protein 3 and fatty acid metabolism. Biochem Soc Trans 29:785–791, 2001.
36. Weigle DS, Selfridge LE, Schwartz MW, Seeley RJ, Cummings DE, Havel PJ, Kuijper JL, BeltrandelRio H. Elevated free fatty acids induce uncoupling protein 3 expression in muscle: a potential explanation for the effect of fasting. Diabetes 47:298–302, 1998.
37. Liu Q, Bai C, Chen F, Wang R, MacDonald T, Gu M, Zhang Q, Morsy MA, Caskey CT. Uncoupling protein-3: a muscle-specific gene upregulated by leptin in *ob/ob* mice. Gene 207:1–7, 1998.
38. Scarpace PJ, Matheny M, Moore RL, Kumar MV. Modulation of uncoupling protein 2 and uncoupling protein 3: regulation by denervation, leptin and retinoic acid treatment. J Endocrinol 164:331–337, 2000.
39. Scarpace PJ, Nicolson M, Matheny M. UCP2, UCP3 and leptin gene expression: modulation by food restriction and leptin. J Endocrinol 159:349–357, 1998.
40. Yasuda T, Masaki T, Kakuma T, Yoshimatsu H. Hypothalamic melanocortin system regulates sympathetic nerve activity in brown adipose tissue. Exp Biol Med (Maywood) 229:235–239, 2004.
41. Tsou K, Khachaturian H, Akil H, Watson SJ. Immunocytochemical localization of pro-opiomelanocortin-derived peptides in the adult rat spinal cord. Brain Res 378:28–35, 1986.
42. Elias CF, Lee C, Kelly J, Aschkenasi C, Ahima RS, Couceyro PR, Kuhar MJ, Saper CB, Elmquist JK. Leptin activates hypothalamic CART neurons projecting to the spinal cord. Neuron 21:1375–1385, 1998.
43. Kishi T, Aschkenasi CJ, Lee CE, Mountjoy KG, Saper CB, Elmquist JK. Expression of melanocortin 4 receptor mRNA in the central nervous system of the rat. J Comp Neurol 457:213–235, 2003.
44. Saper CB, Loewy AD, Swanson LW, Cowan WM. Direct hypothalamo-autonomic connections. Brain Res 117:305–312, 1976.
45. Yoshimatsu H, Egawa M, Bray GA. Sympathetic nerve activity after discrete hypothalamic injections of L-glutamate. Brain Res 601:121–128, 1993.