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Tissue-Specific Effects of Central Leptin on the Expression of Genes Involved in Lipid Metabolism in Liver and White Adipose Tissue

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Leptin reduces adiposity and exerts antisteatotic effects on nonadipose tissues. However, the mechanisms underlying leptin effects on lipid metabolism in liver and white adipose tissue have not been fully clarified. Here, we have studied the effects of central leptin administration on key enzymes and transcription factors involved in lipid metabolism in liver and epididymal adipose tissue. Intracerebroventricular leptin infusion for 7 d did not change leptin plasma levels but decreased triacylglyceride content in liver, epididymal adipose tissue, and plasma. In both tissues this treatment markedly decreased the expression of key enzymes of the *de novo* fatty acid (FA) synthesis such as acetyl-coenzyme A-carboxylase, FA synthase, and stearoyl-coenzyme A desaturase-1, in parallel with a reduction in mRNA expression of sterol regulatory element binding protein-1c in liver and carbohydrate regulatory element binding protein in adipose tissue. In addition, leptin also decreased phosphoenol-pyruvate carboxykinase-C expression in adipose tissue, an enzyme involved in glyceroneogenesis in this tissue. Central leptin administration downregulates delta-6-desaturase expression in liver and adipose tissue, in parallel with the decrease of the expression of sterol regulatory element binding protein-1c in liver and peroxisome proliferator activated receptor α in adipose tissue. Finally, leptin treatment, by regulating adipose triglyceride lipase/hormone sensitive lipase/diacylglycerol transferase 1 expression, also established a new partitioning in the FA-triacylglyceride cycling in adipose tissue, increasing lipolysis and probably the FA efflux from this tissue, and favoring in parallel the FA uptake and oxidation in the liver. These results suggest that leptin, acting at central level, exerts tissue-specific effects in limiting fat tissue mass and lipid accumulation in nonadipose tissues, preventing the development of obesity and type 2 diabetes. (*Endocrinology* 148: 5604–5610, 2007)

LEPTIN, A CYTOKINE produced mainly by the white adipose tissue (WAT), is actively involved in the control of body weight and food intake. Dysregulations of leptin actions are associated with obesity, insulin resistance, and type 2 diabetes. These facts point to leptin and its actions as targets of study to clarify these metabolic disorders and identify potential therapeutic strategies. Several reports have shown that leptin regulates energy homeostasis by controlling peripheral lipid metabolism, and leptin administration reduces triacylglyceride (TAG) stores and promotes fatty acid (FA) oxidation in lean and adipose tissues (1–7). Therefore, it has been postulated that one of the roles of leptin is to reduce lipid accumulation in nonadipose tissues, preventing lipotoxicity (8).

In the liver an acute iv leptin infusion decreases liver TAG secretion, increases hepatic FA oxidation and ketogenesis, and, as a result, decreases liver TAG levels (9). However, an acute intracerebroventricular (icv) leptin administration does not decrease liver TAG levels (9–11). On the other hand, chronic icv leptin treatment decreases TAG content in liver and plasma compared with *ad libitum* fed controls (12), suggesting that leptin, acting at central level, plays an important role depleting TAG levels in this tissue.

In WAT, the effect of leptin on lipid metabolism has not been fully characterized and remains controversial. Thus, adenovirus-induced hyperleptinemia increases the expression of enzymes of FA oxidation such as acyl-coenzyme A oxidase and carnitine palmitoyl transferase (CPT)-1, and depletes the TAG without a concomitant increase in the levels of circulating free FAs (3). This suggests that leptin favored intracellular FA oxidation in adipocytes. Furthermore, treatment of isolated rat adipocytes with leptin up-regulates the expression of acyl-coenzyme A oxidase, CPT-1, uncoupling protein 2, and peroxisome proliferator activated receptor (PPAR) α, all of which are involved in lipid oxidation (13). In addition, *in vivo* (14) and *in vitro* (15) studies indicated a paracrine/autocrine stimulation of lipolysis in rodent adi-

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Abbreviations: ACC, Acetyl-coenzyme A-carboxylase; ATGL, adipose triglyceride lipase; ChREBP, carbohydrate regulatory element binding protein; CPT, carnitine palmitoyl transferase; D6D, delta-6-desaturase; DAGT, diacylglycerol transferase; eWAT, epididymal white adipose tissue; FA, fatty acid; FAS, fatty acid synthase; FAT, fatty acid transporter; HSL, hormone sensitive lipase; icv, intracerebroventricular; MCD, malonyl-coenzyme A decarboxylase; NEFA, non-esterified fatty acid; PEPCK, phosphoenol-pyruvate carboxykinase; PGC-1α, peroxisome proliferator activated receptorγ-coactivator-1α; PPAR, peroxisome proliferator activated receptor; SCD, stearoyl-coenzyme A desaturase; SREBP, sterol regulatory element binding protein; TAG, triacylglyceride; WAT, white adipose tissue.

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pose tissue by leptin, whereas in human adipocytes, *in vitro* leptin did not stimulate lipolysis but inhibited the insulin-induced lipogenesis (16). Other studies suggest that the leptin-induced depletion of WAT is not only due to the mobilization of lipid stores, but also to adipocyte apoptosis (17). In addition, adipocytes incubated with leptin increase the oxidation of exogenous, but not of endogenous FAs, and present a higher TAG breakdown with a concomitant release of FAs from the cells (13).

Most of the aforementioned studies indicate that lipid metabolism could be regulated directly by leptin acting through leptin receptors in peripheral tissues. Nevertheless, much experimental evidence suggests that leptin exerts its effects on lipid metabolism predominantly through the brain, especially through the hypothalamic nuclei, in which the leptin receptor concentration is the highest compared with other tissues (18). Leptin affects appetite by acting through neuroendocrine pathways and autonomic nerves, which transmit leptin signal to the periphery (19). The relative importance of peripheral *vs.* central actions of leptin on metabolic effects of the hormone is not well characterized and needs further investigation.

In the present study, we have investigated the effects of centrally administered leptin on the expression of several enzymes and transcription factors of lipid metabolism in rat liver and epididymal WAT (eWAT). The effect of leptin on TAG FA composition, and stearoyl-coenzyme A desaturase (SCD)-1 and delta-6-desaturase (D6D) expression in both tissues, is also discussed. The possible modulation of centrally administered leptin on the insulin action on plasma non-esterified fatty acid (NEFA) and TAG also has been studied.

Materials and Methods

Animals

The experiments were performed in male, 3-month-old Wistar rats from our in-house colony (Centre of Molecular Biology, Madrid, Spain). Animals were housed in climate-controlled quarters with a 12-h light cycle, and fed *ad libitum* standard laboratory diet and water. They were handled according to the European Union laws and the National Institutes of Health guidelines for animal care. The institutional committee of bioethics approved the experimental procedures, and special care was taken to minimize animal suffering and reduce the number of animals used.

Leptin and insulin administration

The effects of centrally administered leptin on liver and eWAT lipid metabolism have been performed using three groups of animals randomly distributed: 1) rats infused with leptin, 2) rats infused with vehicle and allowed to eat *ad libitum*, and 3) rats infused with vehicle and pair fed to the amount of food consumed by the leptin-infused animals. icv leptin administration was performed as described previously (20). Briefly, rats were anesthetized with ip ketamine/diazepam/atropine used at 50, 4, and 0.2 mg/kg, respectively (Parke-Davis, Roche and Braun, Madrid, Spain) and placed in a stereotaxic frame (David Koppf, Tujunga, CA). An opening in the skull was made with a dental drill at −1.6-mm lateral to the midline and 0.8-mm anterior to bregma. A cannula connected to an osmotic pump (Alzet, Palo Alto, CA) was implanted in the right lateral cerebral ventricle. Osmotic pumps, with a releasing rate of 1 μl/h, were filled with 0.0082 μg/μl (0.2 μg/d) rat leptin (Sigma-Aldrich, St. Louis, MO), or its vehicle (PBS). After 7 d the rats were subjected to an iv insulin (10 IU/kg body weight) (Eli Lilly and Co., Indianapolis, IN) or saline injection for 30 min and killed by decapitation. The liver and eWAT were rapidly dissected for RNA or lipid assays, or frozen in liquid nitrogen and stored at −70 C until use.

Plasma determinations

Blood samples were withdrawn before and after pump implantation. Blood samples were centrifuged, and plasma was frozen at −70 C until use. Plasma leptin and insulin levels were assayed using specific rat kits from SPI-Bio (Montigny le Bretonneux, France). Triacylglycerol (TAG) levels were determined by an enzymatic kit from Stanbio Laboratory (Boerne, TX). Plasma NEFA was measured with an enzymatic kit from WAKO Chemical (Neuss, Germany). Glycerol was quantified by the method of Eggstein and Kuhlmann (21), and glucose was determined using the AmplexRed Glucose/Glucose Oxidase Assay Kit (Molecular Probes, Inc., Eugene, OR).

Tissue TAG and FA composition measurements

Total lipids were extracted from 100 mg tissue (liver or eWAT) by the method of Folch *et al.* (22). Briefly, samples were homogenized in 6 ml chloroform-methanol (2:1). The mixture stood for 1 h, after which, 1.5 ml water was added, and the mixture was centrifuged 10 min at 2000 × *g*. The organic phase was evaporated to dryness under N_2 stream and taken up in chloroform. Fifty microliter aliquots of this organic phase were solubilized by adding a drop of Triton X-100 as described previously (5), and total TAG content was determined using the enzymatic kits from Stanbio Laboratory.

Next, we purified TAG from the total lipids extract using Aminopropyl-bonded (LC-NH2) silica gel cartridges (500-mg matrix) (SUPELCO, Sigma-Aldrich) according to Bodennec *et al.* (23). The collected fractions were again evaporated to dryness under N_2 stream. FAs from hepatic and eWAT TAG were methylated with 5% methanolic HCl. The methyl esters were analyzed using an Agilent 6890 chromatograph equipped with flame ionization detection (Agilent Technologies, Inc., Palo Alto, CA). A fused silica column (length 50 m × internal diameter 0.25 mm) coated with SGL-1000 phase (0.25-μm thickness; Sugerlabor, Madrid, Spain) was used. The carrier gas was helium, at a flow rate through the column of 1 ml/min. The temperature of the injector and detector was set at 250 C and the oven temperature at 210 C. The injection volume was 1 μl. FA methyl esters were identified based on the retention times of known FA standards (Sigma-Aldrich).

RNA extraction and real-time RT-PCR

Total RNA was isolated from liver and eWAT using the TRIzol reagent (Invitrogen Corp., Carlsbad, CA). The cDNA was synthesized from 1.5 μg DNase-treated RNA using the reverse-transcriptase activity from Moloney murine leukemia virus (Life Technologies, Inc.-BRL, Paisley, UK), and p[dN]$_6$ (Boehringer Mannheim, Mannheim, Germany) as a random primer. Relative quantitation of SCD-1, D6D, fatty acid synthase (FAS), acetyl-coenzyme A-carboxylase (ACC)-α, diacylglycerol transferase (DAGT)-1, hormone sensitive lipase (HSL), phosphoenolpyruvate carboxykinase (PEPCK)-C, sterol regulatory element binding protein (SREBP)-1c, carbohydrate regulatory element binding protein (ChREBP), peroxisomes proliferator activating receptor (PPAR) α and γ, PPARγ-coactivator-1α (PGC-1α), malonyl-coenzyme A decarboxylase (MCD), fatty acid transporter (FAT) (FAT/CD36), CPT-1a, and CPT-1b mRNA expression was performed by TaqMan real-time PCR according to the manufacturer's protocol on an ABI PRISM 7500 FAST Sequence Detection System instrument and software (PE Applied Biosystems, Foster City, CA), using Pre-Developed TaqMan Assay Reagents (PE Applied Biosystems). For adipose triglyceride lipase (ATGL), the primers and probe sets were designed using the manufacturer's software and sequences available in GenBank (XM_341960). The probe spanned the boundary between exons 2 and 3.

To standardize the amount of sample cDNA added to the reaction, amplification of endogenous control 18S rRNA was included in separate wells using VIC dye as real-time reporter. The $\Delta\Delta C_T$ method was used to calculate the relative differences between experimental conditions and control groups as fold change in gene expression (24).

Statistics

Data are expressed as mean ± SEM. Statistical analyses were performed using one-way ANOVA (GraphPad Prism version 3.03 software; GraphPad Software, San Diego, CA), followed by the Tukey test.

Results

Biological characteristics of the animals

Before pump implantation, all the animals had similar body weight and fasting plasma parameters. After 7-d central leptin (0.2 μg/d) infusion, no differences were found in plasma leptin levels among the three groups of rats. Leptin-treated rats showed a 30% reduction in food intake and a 5% reduction in body weight. Fasting plasma glucose and glycerol levels remained unchanged in the three groups of animals, whereas plasma insulin decreased significantly in the leptin-treated rats (Table 1).

Central leptin infusion improves the actions of insulin on plasma NEFA, and reduces the TAG contents in liver and eWAT

The main effects of insulin *in vivo* are the stimulation of glucose and FA uptake by the periphery, and the reduction of glucose and TAG output by the liver (25). Euglycemic-hyperinsulinemic clamp experiments have demonstrated that peripheral (26) or intrahypothalamic leptin infusion (27) increases the whole-body insulin sensitivity in terms of glucose metabolism. However, these authors did not detect changes in terms of lipid contents in plasma. Thus, we first analyzed the effects of icv leptin infusion on the ability of insulin to regulate plasma lipid content.

The results of Fig. 1 show that central leptin infusion prevented the decrease in plasma NEFA exerted by the reduction in food intake (Fig. 1A) but decreased the TAG concentrations compared with the pair-feeding group (Fig. 1B). Upon acute iv insulin stimulation, in the *ad libitum*-fed control rats, NEFA levels were reduced by 25% (Fig. 1, A and C), and TAG contents decreased by 30% (Fig. 1, B and D). In leptin-treated rats, insulin did not further reduce the already low levels of plasma TAG. However, leptin acted synergistically with insulin in decreasing markedly NEFA levels in plasma (55%) (Fig. 1, A and C).

According to its well-known lipostatic function, central leptin infusion significantly reduced TAG levels (Fig. 2B) in eWAT, compared with the vehicle-infused pair-fed group of rats. In the liver, leptin reduced the hepatic TAG content (Fig. 2A) compared with the vehicle-infused *ad libitum*-fed rats, but not to the pair-fed animals.

Central leptin regulates the expression of transcription factors and key enzymes involved in lipid metabolism in liver and eWAT

Tables 2 and 3 summarize the effects of central leptin administration on different key enzymes and transcription factors involved in lipid metabolism in liver and eWAT, respectively. We analyzed the expression of transcription factors involved in lipid synthesis such as SREBP-1c, ChREBP, or PPARγ and the transcription factors involved in lipid oxidation: PPARα and PGC-1α. As shown in Table 2, SREBP-1c mRNA expression increased during the pair feeding and was down-regulated by central leptin treatment in the liver. The same profile was observed with ChREBP mRNA expression in eWAT, with no effects on SREBP-1c gene expression in this tissue (Table 3). PPARγ mRNA levels were analyzed only in eWAT, in which it is mainly expressed. Surprisingly, leptin enhanced the up-regulation of PPARγ mRNA levels already stimulated by the pair feeding in eWAT (Table 3). On the other hand, in liver, central leptin infusion increased the expression of PPARα and PGC-1α compared with the pair-feeding group (Table 2), whereas in eWAT the hormone treatment up-regulated the mRNA levels of PGC-1α but decreased the PPARα mRNA expression when compared with the pair-feeding rats (Table 3).

In agreement with the observed changes in hepatic SREBP-1c expression in liver, central leptin blunted the up-regulation of the mRNA levels of ACC, FAS, and SCD-1 elicited by the pair feeding (Table 2), suggesting a decrease in *de novo* FA synthesis in this tissue of leptin-treated rats. D6D catalyzes the rate-limiting step in the conversion of linoleic acid (n6) and α-linolenic acid (n3), into arachidonic acid (n6) and eicosapentaenoic acid (n3), respectively, therefore having also an important role in the synthesis of many molecules involved in cell signaling. As shown in Table 2, leptin decreases the expression of this key enzyme and counterbalances the up-regulation induced by the decrease in food intake appreciated in the pair-feeding group.

In eWAT, the effects of centrally administered leptin on the expression of lipogenic enzymes are similar to those observed in liver. However, in marked contrast to leptin-mediated hepatic SREBP-1c down-regulation (Table 2), it has to be pointed out that the reduction on the expression of the same lipogenic genes as well as that of the glyceroneogenic enzyme PEPCK-C caused by central leptin occurs without changes in SREBP-1c expression (Table 3).

We next examined the expression levels of DAGT-1 and ATGL in eWAT, and we observed that leptin enhanced the expression of ATGL and DAGT-1 elicited by the pair feeding in eWAT (Table 3). This, together with a higher expression of HSL in leptin-treated rats, compared with the *ad libitum*-fed controls, suggests an increased basal lipolysis in this group of animals.

Central leptin infusion up-regulated the mRNA expres-

TABLE 1. Effects of central leptin administration on the biological characteristics of the animals

Treatment	SS	PF	Leptin
Initial body weight (g)	353 ± 10^a	372 ± 20^a	359 ± 13^a
Final body weight (g)	380 ± 13^a	359 ± 11^b	344 ± 8^b
Daily food intake (g)	19 ± 1^a	13 ± 1^b	13 ± 1^b
Plasma glucose (mM)	6.5 ± 0.8^a	5.9 ± 0.4^a	5.9 ± 0.6^a
Plasma glycerol (mg/dl)	61 ± 5^a	59 ± 5^a	62 ± 3^a
Plasma insulin (ng/ml)	1.6 ± 0.5^a	1.4 ± 0.1^a	0.7 ± 0.1^b
Plasma leptin (ng/ml)	5.9 ± 0.5^a	5.4 ± 1^a	5.2 ± 0.8^a

Body weight and food intake were measured daily during the experiment. After 7-d central leptin (Lep) 0.2 μg/d or vehicle [vehicle-infused rats fed *ad libitum* (SS) or vehicle-infused pair-fed rats (PF)] infusion, glucose, glycerol, insulin, and leptin were assessed in plasma. Results are the mean ± SEM; n = 6–10 rats per group, made in triplicate. Values that do not share the *same superscript letter* are significantly different ($P < 0.05$).

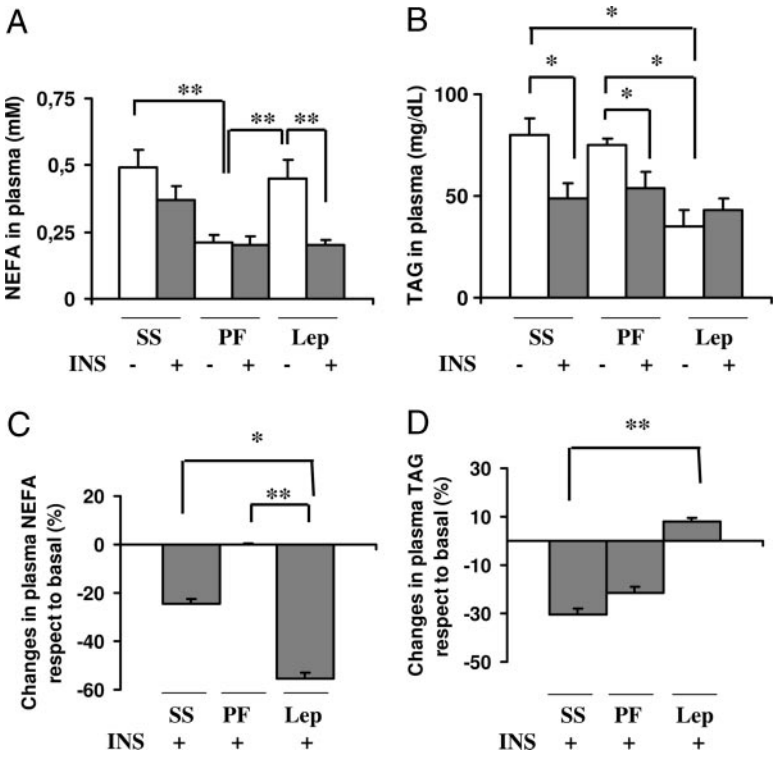

FIG. 1. Central leptin infusion improves insulin lowering effects on plasma NEFA. After 7-d central leptin (Lep) 0.2 μg/d or vehicle [vehicle-infused rats fed *ad libitum* (SS) or vehicle-infused pair-fed rats (PF)] infusion, rats were injected iv with insulin (INS) (10 IU/kg body weight) or vehicle. At 30 min after injection, plasma NEFA (A) and TAG (B) were measured. C, Changes in plasma NEFA: percent respect basal value (after insulin infusion). D, Changes in plasma TAG: percent respect basal value (after insulin infusion). Results are the mean ± SEM; n = 6–8 rats per group, made in triplicate. *, $P < 0.05$. **, $P < 0.01$.

sion of FAT/CD36 in liver (Table 2), but not in eWAT (Table 3), suggesting an increased FA uptake only in liver. In addition, in both tissues the pair feeding up-regulated the CPT-1 and MCD mRNA levels (Tables 2 and 3), compared with the vehicle-infused rats fed *ad libitum*, and central leptin augmented this effect.

Effects of central leptin on liver and eWAT TAG FA composition

As previously shown, central leptin infusion down-regulated the mRNA levels of SCD-1 and D6D in liver (Table 2) and eWAT (Table 3). To determine if these changes in the expression of both desaturases were reflected in the FA composition of TAG in both tissues, TAGs were purified from total lipid extracts, and their FAs were analyzed by gas chromatography.

In liver from leptin-treated rats, the TAG FA profile increased its proportion of palmitic and stearic, and decreased the one of oleic acid (Table 4). Consequently, the SCD-1 index was reduced. Although the proportion of linoleic acid, the substrate of D6D, increased, the D6D index did not change because the proportion of arachidonic acid was also augmented after the leptin treatment when compared with the pair-fed rats. Interestingly, central leptin infusion for 7 d did not alter the TAG FA relative abundance in eWAT, in agreement with the lower lipogenic capacity of this tissue compared with the liver.

Discussion

The data presented here demonstrate that icv leptin infusion exerts tissue-specific effects on the expression of key enzymes and transcription factors involved in lipid metabolism in liver and eWAT. As a result, central leptin affects the TAG FA composition in liver, but not in eWAT. The fact that plasma leptin does not increase after icv leptin administration excludes that the observed effects by this treatment were due to peripheral actions of the hormone. Thus, leptin, acting through hypothalamic neural circuits and the autonomic nervous system, represses the expression of genes involved in *de novo* synthesis of FAs such as ACC and FAS in both liver and eWAT, which

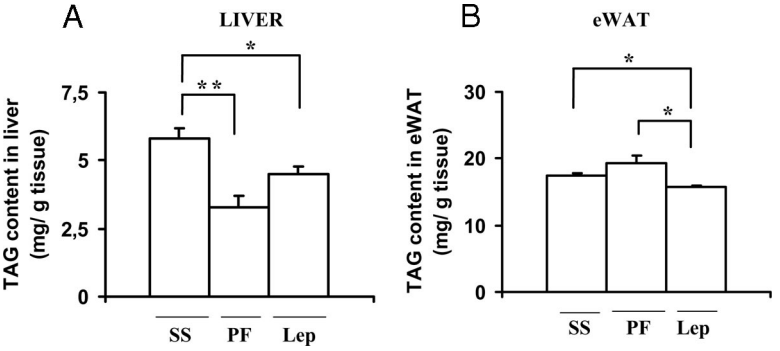

FIG. 2. Central leptin effects on TAG content in liver and eWAT. After 7-d central leptin (Lep) 0.2 μg/d or vehicle [vehicle-infused rats fed *ad libitum* (SS) or vehicle-infused pair-fed rats (PF)] infusion, TAG content was measured in liver (A) and eWAT (B). Results are the mean ± SEM; n = 10–16 rats per group, made in triplicate. *, $P < 0.05$. **, $P < 0.01$.

TABLE 2. Changes in mRNA expression in liver after icv leptin treatment using 18S rRNA as the invariant control

Gene	SS	PF	Lep
		Transcription factor of lipid synthesis	
SREBP-1c	1.0 ± 0.4^a	6.4 ± 0.7^b	1.1 ± 0.3^a
		Transcription factors of lipid oxidation	
PGC-1α	1.0 ± 0.05^a	0.5 ± 0.4^b	2.0 ± 0.7^c
PPARα	1.0 ± 0.2^a	3 ± 1^b	7 ± 1^c
		FA *de novo* synthesis and desaturation	
ACC	1.0 ± 0.07^a	23.2 ± 0.6^b	2.6 ± 0.6^c
FAS	1.0 ± 0.1^a	12 ± 2^b	0.4 ± 0.1^c
SCD-1	1.0 ± 0.2^a	169 ± 60^b	0.5 ± 0.5^a
D6D	1.0 ± 0.4^a	23 ± 2^b	12.8 ± 0.5^c
		FA uptake and β-oxidation	
FAT/CD36	1.0 ± 0.2^a	0.6 ± 0.2^a	2.3 ± 0.2^b
CPT-1a	1.0 ± 0.08^a	1.4 ± 0.09^b	3.1 ± 0.5^c
MCD	1.0 ± 0.2^a	1.9 ± 0.5^a	1.9 ± 0.5^a

Values are the means ± SEM; n = 4–6 rats per group, made in triplicate. Values that do not share the *same superscript letter* are significantly different ($P < 0.05$, one-way ANOVA followed by Tukey test). Lep, Leptin-infused rats; PF, vehicle-infused pair-fed rats; SS, vehicle-infused rats fed *ad libitum*.

agrees well with previous reports in adenovirus-induced hyperleptinemic rats (1, 2). Central leptin also down-regulates SCD-1 and D6D mRNA expression in rat liver and eWAT, when compared with pair-fed animals. In agreement with previously published data (28), our results suggest that by reducing SCD-1 expression, the FA synthesis, and concomitantly, the synthesis of TAG are probably decreased. In addition, the up-regulation of CPT-1 by central leptin infusion, a key enzyme of β-oxidation, may contribute to reduced hepatic and plasma TAG content.

Although considerable experimental data show that leptin decreases SCD-1 gene expression and/or enzyme activity (5, 29), there was no evidence reporting the relationship between leptin and D6D. Most studies on D6D have been performed in liver (30, 31), whereas data about D6D in WAT have been scarce (32, 33). Here, we provide evidence for the first time of central leptin-mediated down-regulation of D6D mRNA expression in peripheral tissues, such as liver and eWAT.

The expression profiles of ACC, FAS, and SCD-1 parallel the expression of the lipogenic transcription factors SREBP-1c in liver and of ChREBP in eWAT, suggesting that central leptin may impair lipogenesis in liver and eWAT, acting in a tissue-specific manner, through different lipogenic transcription factors. In the adipose tissue, ChREBP seems to play an important role in the regulation of fuel metabolism. Decreased epididymal fat weight in addition to reduced hepatic lipogenesis was recently reported by Iizuka *et al.* (34) in the double-deficient mice *ob*/*ob*-ChREBP−/−. In liver, the decrease in SREBP-1c mRNA

TABLE 3. Changes in mRNA expression in eWAT after icv leptin treatment using 18S rRNA as the invariant control

Gene	SS	PF	Lep
		Transcription factors of lipid synthesis	
SREBP-1c	1.0 ± 0.1^a	1.2 ± 0.3^a	1.0 ± 0.3^a
CHREBP	1.0 ± 0.07^a	2.2 ± 0.3^b	1.1 ± 0.2^a
PPARγ	1.0 ± 0.1^a	1.7 ± 0.1^b	2.6 ± 0.3^c
		Transcription factors of lipid oxidation	
PGC-1α	1.0 ± 0.09^a	2.0 ± 0.08^b	4.0 ± 0.8^c
PPARα	1.0 ± 0.05^a	1.9 ± 0.3^b	1.1 ± 0.05^a
		FA *de novo* synthesis and desaturation	
ACC	1.0 ± 0.2^a	2.9 ± 0.7^b	1.3 ± 0.3^a
FAS	1.0 ± 0.8^a	4 ± 1^b	0.4 ± 0.2^a
SCD-1	1.0 ± 0.04^a	4.1 ± 0.4^b	0.3 ± 0.04^c
D6D	1.0 ± 0.2^a	1.8 ± 0.5^b	0.9 ± 0.3^a
		Glyceroneogenesis	
PEPCK-c	1.0 ± 0.2^a	1.4 ± 0.1^b	1.0 ± 0.08^a
		FA uptake and β-oxidation	
FAT/CD36	1.0 ± 0.2^a	0.9 ± 0.09^a	1.0 ± 0.03^a
CPT-1b	1.0 ± 0.1^a	2.8 ± 0.5^b	5 ± 2^c
MCD	1.0 ± 0.05^a	1.6 ± 0.1^b	2.9 ± 0.2^c
		Lipolysis and esterification	
ATGL	1.0 ± 0.05^a	1.3 ± 0.1^b	1.9 ± 0.09^c
HSL	1.0 ± 0.07^a	2.6 ± 0.3^b	2.8 ± 0.7^b
DAGT-1	1.0 ± 0.08^a	1.5 ± 0.2^b	2.5 ± 0.2^c

Values are the means ± SEM; n = 4–6 rats per group, made in triplicate. Values that do not share the *same superscript letter* are significantly different ($P < 0.05$, one-way ANOVA followed by Tukey test). Lep, Leptin-infused rats; PF, vehicle-infused pair-fed rats; SS, vehicle-infused rats fed *ad libitum*.

TABLE 4. Central leptin infusion changes the TAG FA relative abundance in liver, but not in eWAT

FA (%)	LIVER			WAT		
	SS	PF	Leptin	SS	PF	Leptin
14:0	0.6 ± 0.03^{a}	1.0 ± 0.1^{b}	0.6 ± 0.03^{a}	1.4 ± 0.05^{a}	1.5 ± 0.05^{a}	1.4 ± 0.04^{a}
16:0	38 ± 1^{a}	27 ± 2^{b}	40 ± 2^{a}	24 ± 0.3^{a}	25 ± 0.6^{a}	25 ± 0.5^{a}
16:1	3.3 ± 0.3^{a}	4.2 ± 0.8^{a}	2.4 ± 0.1^{a}	6.7 ± 0.2^{a}	7.4 ± 0.5^{a}	5.8 ± 0.5^{a}
18:0	5.6 ± 0.7^{a}	$3.7 \pm 0.2^{a,b}$	$8 \pm 1^{a,c}$	2.7 ± 0.07^{a}	2.6 ± 0.09^{a}	2.8 ± 0.1^{a}
18:1	20 ± 0.7^{a}	39 ± 2^{b}	16 ± 0.3^{a}	30 ± 0.4^{a}	30 ± 0.3^{a}	29 ± 0.2^{a}
18:2	28 ± 0.8^{a}	19 ± 2^{b}	31 ± 2^{a}	31 ± 0.6^{a}	29 ± 1^{a}	33 ± 1^{a}
18:3	1.2 ± 0.1^{a}	0.7 ± 0.1^{a}	1.1 ± 0.1^{a}	1.4 ± 0.05^{a}	1.5 ± 0.04^{a}	1.4 ± 0.04^{a}
20:1	0.2 ± 0.03^{a}	0.5 ± 0.2^{b}	0.2 ± 0.02^{a}	0.2 ± 0.01^{a}	0.2 ± 0.01^{a}	0.2 ± 0.02^{a}
20:4	7.3 ± 0.9^{a}	4.6 ± 0.8^{b}	7 ± 1^{a}	1.0 ± 0.07^{a}	0.9 ± 0.1^{a}	0.9 ± 0.01^{a}
(16:1/16:0) × 100 SCD-1 index	10 ± 2^{a}	$16 \pm 3^{a,b}$	$6.0 \pm 0.5^{a,c}$	26 ± 1^{a}	29 ± 1^{a}	24 ± 2^{a}
(20:4/18:2) × 100 D6D index	26 ± 6^{a}	26 ± 6^{a}	22 ± 2^{a}	3.3 ± 0.2^{a}	3.2 ± 0.4^{a}	2.9 ± 0.2^{a}

After 7-d central leptin (Lep) 0.2 μg/d or vehicle [vehicle-infused rats fed *ad libitum* (SS) or vehicle-infused pair-fed rats (PF)] infusion, TAGs were purified from liver or eWAT total lipid extracts, and TAG FA relative abundance was assessed by gas chromatography. Results are the mean ± SEM; n = 6–8 rats per group, made in triplicate. Values that do not share the *same superscript letter* are significantly different ($P < 0.05$).

levels could be mediated by the increased gene expression of PPARα because the latter has blocked the activity of liver X receptor on the SREBP-1c promoter, thereby preventing SREBP-1c transcription (35).

It has been reported that liver D6D mRNA expression can be activated by SREBP-1c or PPARα, depending on the metabolic context (32, 36). The pair feeding elicited an up-regulation of SREBP-1c and PPARα mRNA expression in the liver, which could respond for the stimulation of the D6D expression under these conditions. However, central leptin infusion promoted an increase of hepatic PPARα, but a down-regulation of SREBP-1c and D6D gene expression compared with the pair-fed rats. Together, these data suggest that hepatic D6D would be regulated by central leptin mainly through SREBP-1c. On the other hand, in eWAT, neither the pair feeding nor central leptin seems to regulate SREBP-1c mRNA expression, whereas the pattern of PPARα mRNA expression parallels the expression of D6D, suggesting that, in this tissue, central leptin regulates D6D gene expression mainly through PPARα.

Consistent with the SCD-1 and D6D expression data, the proportion of oleic acid decreases in the TAG FA content in liver, whereas the relative abundance of linoleic acid increases. Contrasting with the liver, the changes in desaturase expression do not seem to be reflected in the TAG FA profile in eWAT. This could be explained by the lower lipogenic capacity of eWAT compared with the liver. In addition, the decreased expression of PECK-C in eWAT, an enzyme involved in glyceroneogenesis, and the significant reduction in lipoprotein lipase activity reported in eWAT after central leptin administration (12) could reduce the incorporation of exogenous FA to TAG in this tissue, and then contribute to explaining the unaltered TAG-FA composition of the eWAT after the central leptin treatment. Furthermore, central leptin administration impairs the insulin-stimulated glucose uptake in eWAT (27) (unpublished observations), which would also contribute to reducing the glycerol availability and, thus, *de novo* TAG synthesis in this tissue.

According to our gene expression data, the lipolysis in eWAT of leptin-infused rats seems to be mediated by ATGL and HSL, and the re-esterification by DAGT-1. ATGL is a triglyceride-specific lipase recently identified, and only studied in mouse and humans (37, 38). The different substrate preferences between ATGL and HSL suggest a serial and coordinated action of these two enzymes in FA store mobilization, with ATGL providing diacylglyceride substrate for HSL. Although the mechanisms involved in the regulation of ATGL remain unclear, several studies have shown that the expression of this protein is sensitive to the nutritional status of the organism. Thus, ATGL expression is induced in response to fasting (39), which agrees with the increased expression of ATGL mRNA in the eWAT of pair-fed rats. Moreover, our data, which demonstrate an up-regulation of ATGL in eWAT by central leptin, are consistent with the findings reported by Villena *et al.* (39), who showed that ATGL mRNA levels are very low in obese rodent models, in which leptin levels are low (*ob/ob* mice), or the leptin receptor is dysfunctional (*db/db* mice).

Although we have not directly measured the lipolysis and glyceroneogenesis in eWAT, according to the gene expression data reported here, an elevated lipolysis and suppression of glyceroneogenesis could be the explanation of the plasma FA levels observed in leptin-treated rats and of the decrease in eWAT intracellular TAG levels, compared with the pair-fed group. Moreover, due to the central leptin-mediated up-regulation of the hepatic FAT/CD36 mRNA levels, it is tempting to speculate that the FA uptake may be increased in the liver. Under these conditions, the liver may play an important role by taking up and oxidizing this extra NEFA flux, probably released from the eWAT, because in the leptin-infused rats compared with the pair-fed ones, hepatic FAT/CD36 and CPT-1 mRNA levels are increased, possibly through a PPARα-mediated mechanism, as deduced by the changes in expression of this transcription factor described here. This, together with a lower expression of ACC, the enzyme producing malonyl-coenzyme A, in the liver of leptin-treated rats, would favor the FA β-oxidation in this tissue. As a result, intrahepatic TAG levels are reduced by central leptin treatment.

In conclusion, the results presented here demonstrate that central leptin administration, by regulating in a different way the gene expression of some transcription factors such as SREBP-1c and PPARα, exerts tissue-specific effects on lipid metabolism in liver and eWAT, and changes the TAG FA composition in liver, but not in eWAT. Therefore, it is plausible that

leptin, acting at a central level, probably leads to the down-regulation of the *de novo* lipogenesis in both tissues, increases lipolysis in eWAT through the ATGL/HSL/DAGT1 pathway and probably the FA release from this tissue, and promotes a proportional FA uptake and oxidation mainly in the liver. Nevertheless, these suggestions need further work before a final conclusion may be drawn.

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