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Regional differences in the expression of genes involved in lipid metabolism in adipose tissue in response to short- and medium-term fasting and refeeding

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Abstract

The aim of this study was to analyze regional differences in the time-course response to fasting and refeeding in the expression of genes involved in lipid metabolism in retroperitoneal, mesenteric and inguinal adipose tissue. Rats were studied under different feeding conditions: feeding state; after 4, 8 or 24 h of fasting; and after 3 h of refeeding following 8 h of fasting. The expression of lipogenesis-related genes decreased by fasting in adipose tissue, and the retroperitoneal depot showed the fastest response: mRNA levels of peroxisome proliferator-activated receptor gamma 2 (PPARγ2) decreased after 4 h of fasting and those of sterol regulatory element binding protein 1c (SREBP1c), fatty acid synthase (FAS), GPAT and glucose transporter 4 (GLUT4) decreased after 8 h. In the inguinal depot, mRNA levels of SREBP1c, acetyl-coenzyme A carboxylase alpha, FAS and lipoprotein lipase decreased after 8 h of fasting, while in the mesenteric depot, only GLUT4 and FAS mRNA levels decreased after 8 and 24 h, respectively. Concerning lipolytic and fatty acid oxidation genes, only adipose triglyceride lipase and carnitine palmitoyltransferase 1a expression increased after 24 h of fasting in the retroperitoneal depot. Three hours of refeeding restored the expression of the lipogenic transcription factors PPARγ2 and SREBP1c in the retroperitoneal depot and of PPARγ2 in the inguinal depot. This period of refeeding was ineffective in changing the expression of genes related with lipid mobilization and fatty acid oxidation, except hormone-sensitive lipase, whose expression decreased in the mesenteric depot. It is suggested that different regulations of the expression of genes related with lipid metabolism between internal and subcutaneous depots to feeding and fasting conditions are site-specific metabolic features of white adipose tissue.

Keywords: Lipogenesis; Lipolysis; White adipose tissue depots; Food deprivation; Gene expression

1. Introduction

Adipose tissue is recognized to have different functions that are important in the regulation of energy balance and substrate metabolism. One main role of this tissue is to act as a reservoir for fuel storage in the form of triacylglycerides as well as to mobilize this fuel when needed, for example, during fasting or under situations with elevated energy demands [1]. Both fat storage and fat mobilization processes occur normally under the habitual food intake/fasting patterns of feeding and allow the maintenance of energy homeostasis [2,3].

Under fasting conditions, increased lipolysis of triacylglycerides stored in the adipose tissue provides nonesterified (or free) fatty acids (NEFA) to be used as an energy source by other tissues [4] (see Fig. 1). The breakdown of triacylglycerides is known to be mediated by hormone-sensitive lipase (HSL) and the more recently discovered adipose triglyceride lipase (ATGL) [5], also called desnutrin [6]. Although part of released fatty acids are oxidized in the adipose tissue, they are mainly conducted to the liver and metabolized via the mitochondrial and peroxisomal β-oxidation enzyme systems [7]. Carnitine palmitoyltransferase 1a (CPT1) and acyl-

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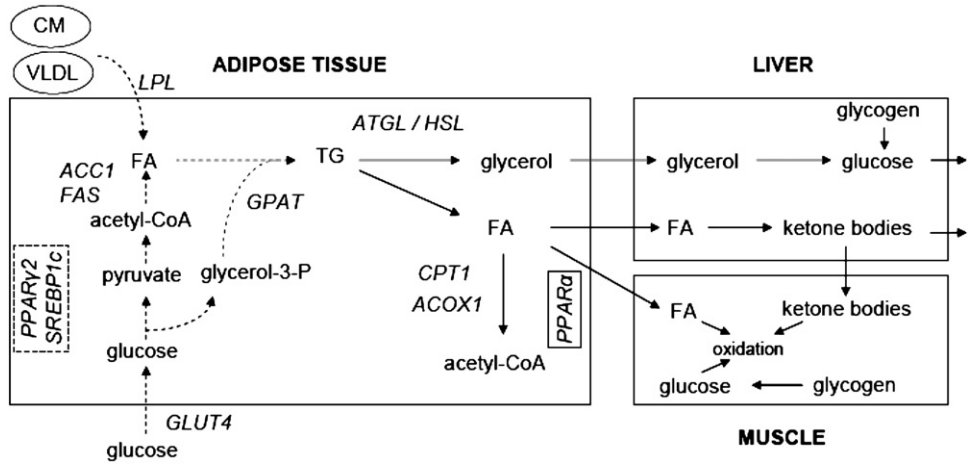

Fig. 1. Schematic view of the interplay of the adipose tissue, liver and skeletal muscle under fasting conditions (solid lines). Main pathways under feeding conditions in adipose tissue are also indicated with broken lines. Key ligand-activated transcription factors regulating lipogenesis (PPARγ and SREBP1c) and fatty acid oxidation (PPARα) and main enzymes and transporters related with lipid metabolism in the adipose tissue are indicated. Abbreviations: ACC1, acetyl-coenzyme A carboxylase alpha; ACOX1, acyl-coenzyme A oxidase 1; ATGL, adipose triglyceride lipase; CM, chylomicrons; CPT1, carnitine palmitoyltransferase 1a; FA, fatty acids; FAS, fatty acid synthase; GLUT4, glucose transporter 4; GPAT, glycerol-3-phosphate acyltransferase; HSL, hormone-sensitive lipase; LPL, lipoprotein lipase; PPARα, peroxisome proliferator-activated receptor alpha; PPARγ2, peroxisome proliferator-activated receptor gamma 2; SREBP1c, sterol regulatory element binding protein 1c; TG, triacylglycerides; and VLDL, very low density lipoproteins.

coenzyme A oxidase 1 (ACOX1) — a target gene of peroxisome proliferator-activated receptor alpha (PPARα) — are key enzymes of mitochondrial and peroxisomal oxidation of fatty acids, respectively. On the other hand, while lipolysis is stimulated, fasting conditions are associated with decreased lipogenesis, and this adaptation appears to involve changes in the two main lipogenic transcription factors, namely, peroxisome proliferator-activated receptor gamma (PPARγ) and sterol regulatory element binding protein 1c (SREBP1c) [8,9]. PPARγ is a member of the PPAR family — with two protein isoforms, PPARγ1 (expressed in a wide variety of tissues) and PPARγ2 (preferentially expressed in adipose tissue), encoded by the same gene — which plays a role in glucose metabolism, lipid metabolism and cell cycle and is an adipocyte differentiation factor (see Ref. [10]). In addition to its stimulatory effects on pre-adipocyte differentiation, activation of PPARγ promotes the storage of fatty acids in mature adipocytes by acting on several steps, including the stimulation of lipoprotein lipase (LPL) [1,8]. In addition, PPARγ has been shown to be capable of activating glucose transporter 4 (GLUT4) expression, which could contribute to increased fatty acid synthesis from glucose [11]. The SREBP1c factor is involved in the regulation of the expression of key genes of lipid and glucose metabolism such as fatty acid synthase (FAS), acetyl-coenzyme A carboxylase (ACC) and hexokinase II [9,12,13]. Interestingly, SREBP1c has also been shown to regulate PPARγ expression in cultured adipocytes [14]; moreover, the expression of the activated form of SREBP1c leads to the production of ligands for PPARγ [15], suggesting that the actions of both adipogenic factors are coordinated. The expression of SREBP1c has been found to be regulated by insulin; in fact, the enhanced synthesis of fatty acids in response to insulin is critically dependent on the transcription factor SREBP1c [16]. Thus, this factor has been proposed to be directly involved in the integration of nutritional changes and insulinemia variations at the level of gene transcription [12,13].

In mammals, adipose tissue is distributed in different anatomical sites, both subcutaneously and internally, which have different biological functions and different metabolic activities [1,17,18]. Fat accumulation is known to be accompanied by several metabolic disorders, although the distribution of body fat appears to be even more important than the total amount of fat [17,19,20]. In particular, visceral adiposity has been strongly linked to insulin resistance, type 2 diabetes, hypertension and dyslipidemia, whereas such associations seem to be much less consistent with respect to subcutaneous fat mass [21,22]. Hence, accumulation of fat in the abdominal visceral region appears to provide the greatest health risk, although the underlying mechanisms of this association are not completely resolved [22].

The adverse metabolic impact of visceral fat has been attributed to distinct biological properties of its adipocytes compared with other adipose tissue depots [1,21]. Indeed, regional variations in the metabolic activity of fat cells have been observed, especially in regard to lipolysis regulation. Visceral adipose tissue has a larger amount of beta-adrenergic receptors, and this can explain its higher lipolytic activity [23]. In fact, the mobilization of visceral fat pads has been shown to be greater than that of subcutaneous depots; thus, during starvation or caloric restriction [24], visceral adipose tissue may release proportionally more fatty acids than subcutaneous adipose tissue [1,24,25]. In addition, visceral adipocytes may have

higher metabolic activity compared with subcutaneous adipocytes, since insulin-stimulated glucose uptake has been found to be higher in visceral compared with subcutaneous fat [26]. Excess visceral fat would lead to greater fatty acid delivery to the liver, and this would impair insulin clearance and action and increase glucose and very low density lipoprotein (VLDL) output from the liver [27,28]. Regardless of the site of origin, high levels of NEFA in the circulation will also impair peripheral glucose disposal [29,30], and thus, NEFA will promote insulin resistance, hyperinsulinemia and hyperglycemia.

Given the clinical importance of differences in regional fat distribution, information regarding possible site-specific differences in the expression of genes involved in lipogenesis and lipolysis could provide insights into the capacity to regulate body fat content and distribution and body weight maintenance, which is of great interest in the prevention/treatment of obesity and its metabolic-related disorders. Here, we have characterized the time-course response to a short- to medium-term food deprivation period and refeeding in the expression of selected genes encoding proteins involved in lipid metabolism in different fat depots, including two internal depots (retroperitoneal and mesenteric) and the inguinal (subcutaneous) depot. Changes in the expression of some genes involved in lipid metabolism in the retroperitoneal depot as an effect of fasting have been previously reported in the same cohort of animals [31], but here we have extended the study to other adipose depots to assess possible differences according to the anatomical localization. The genes studied, selected according to their metabolic significance as introduced before, were the key ligand-activated transcription factors SREBP1c, PPARα and PPARγ2, as well as other selected genes related with lipogenesis [ACC1, FAS, glycerol-3-phosphate acyltransferase (GPAT), LPL and GLUT4], lipolysis (HSL and ATGL) and fatty acid oxidation (CPT1 and ACOX1).

2. Materials and methods

2.1. Animals

Three-month-old male Wistar rats (Charles River Laboratories España, SA, Barcelona, Spain), housed at 22°C with a 12-h light/dark period (lights on from 0800 to 2000 h) and fed with a standard chow diet (Panlab, Barcelona, Spain), were used. Rats were housed individually in cages with wire-mesh bottoms in order to prevent coprophagia and were randomly distributed into five different feeding groups (n=6–8): a control group (animals provided with ad libitum access to chow diet), three fasted groups (animals deprived of food for 4, 8 or 24 h before sacrifice) and a refed group (animals fasted for 8 h and allowed free access to chow diet for 3 h). All animals had free access to tap water. All animals were killed by decapitation during the first hour after the beginning of the light cycle, except for the animals in the refed group, which were killed 3 h after the beginning of the light cycle. To design the fasting protocol, we considered the fact that rats eat most of their daily food intake at night [32]; thus, the 4 and 8 h of food deprivation were during the dark cycle and just before the beginning of the light cycle.

After killing the animals, the retroperitoneal, mesenteric and inguinal white adipose tissue depots were rapidly removed, weighed and frozen in liquid nitrogen and stored at −70°C until RNA analysis. Blood was collected in a heparinized glass and centrifuged at 700×g for 10 min to obtain the plasma.

2.2. Quantification of circulating glucose, insulin, NEFA and β-hydroxybutyrate concentration

Blood glucose concentration was measured by Accu-Check Glucometer (Roche Diagnostics, Barcelona, Spain). Plasma insulin concentration was measured using a rat insulin enzyme-linked immunosorbent assay kit (DRG Instruments GmbH, Marburg, Germany) following standard procedures. Commercial enzymatic colorimetric kits were used for the determination of plasma NEFA (Wako Chemicals GmbH, Neuss, Germany) and 3-hydroxybutyrate levels (β-HBA Procedure No. 310-UV, Sigma Diagnostics).

2.3. RNA extraction

Total RNA was extracted from different white adipose tissue depots by Tripure Reagent (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer's instructions. Isolated RNA was quantified using the NanoDrop ND-1000 spectrophotometer (NadroDrop Technologies Inc., Wilmington, DE, USA), and its integrity was confirmed using agarose gel electrophoresis.

2.4. Real-time quantitative polymerase chain reaction analysis

Real-time polymerase chain reaction (PCR) was used to measure mRNA expression levels of SREBP1c, PPARα, PPARγ2, acetyl-coenzyme A carboxylase alpha (ACC1), FAS, GPAT, LPL, GLUT4, HSL, ATGL, CPT1 and ACOX1 in different white adipose tissue depots.

Total RNA (0.25 μg, in a final volume of 12.5 μl) was denatured at 65°C for 10 min and then reverse transcribed to cDNA using MuLV reverse transcriptase (Applied Biosystems, Madrid, Spain) at 20°C for 15 min, 42°C for 30 min, with a final step of 5 min at 95°C in a PerkinElmer 9700 Thermal Cycler (PerkinElmer, Wellesley, MA). Real-time PCR was completed using the LightCycler System with SYBR Green I sequence nonspecific detection (Roche Diagnostics GmbH). Primers for the different genes are described in Table 1. All primers were obtained from Bonsai Technologies Group, SA (Madrid, Spain).

Each PCR was performed in a total volume of 8 μl, made from diluted (1/50) cDNA template (2 μl), forward and reverse primers (1 μM each) and SYBR Green I master mix (1.8 μl, including Taq polymerase, reaction buffer, $MgCl_2$,

Table 1
Nucleotide sequences of primers and cycling conditions used for PCR amplification

Gene	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)	Cycling conditions	Amplicon size (bp)
ACC1	TGCAGGTATCCCCACTCTTC	TTCTGATTCCCTTCCCTCCT	95°C for 2 s 60°C for 6 s 72°C for 10 s	212
ACOX1	CACGCAATAGTTCTGGCTCA	ACCTGGGCGTATTTCATCAG	95°C for 2 s 60°C for 6 s 72°C for 10 s	221
ATGL	TGTGGCCTCATTCCTCCTAC	AGCCCTGTTTGCACATCTCT	95°C for 2 s 60°C for 6 s 72°C for 10 s	271
CPT1	GCTCGCACATTACAAGGACAT	TGGACACCACATAGAGGCAG	95°C for 2 s 60°C for 6 s 72°C for 10 s	250
FAS	CGGCGAGTCTATGCCACTAT	ACACAGGGACCGAGTAATGC	95°C for 2 s 60°C for 6 s 72°C for 10 s	222
GLUT4	GGCATGGGTTTCCAGTATGT	GCCCCTCAGTCATTCTCATG	95°C for 2 s 60°C for 6 s 72°C for 10 s	233
GPAT	CAGCGTGATTGCTACCTGAA	CTCTCCGTCCTGGTGAGAAG	95°C for 2 s 60°C for 6 s 72°C for 10 s	194
HSL	TCACGCTACATAAAGGCTGCT	AGTTCCCTCTTTACGGGTGG	95°C for 2 s 60°C for 6 s 72°C for 7s	169
LPL	TATGGCACAGTGGCTGAAAG	CTGACCAGCGGAAGTAGGAG	95°C for 2 s 60°C for 6 s 72°C for 8s	157
PPARα	TGTCGAATATGTGGGGACAA	AAACGGATTGCATTGTGTGA	95°C for 2 s 60°C for 6 s 72°C for 8s	215
PPARγ2	GATCCTCCTGTTGACCCAGA	TCAAAGGAATGGGAGTGGTC	95°C for 2 s 60°C for 6 s 72°C for 10 s	164
SREBP1c	AGCCATGGATTGCACATTTG	GGTACATCTTTACAGCAGTG	95°C for 2 s 60°C for 6 s 72°C for 10 s	260
18S	CGCGGTTCTATTTTGTTGGT	AGTCGGCATCGTTTATGGTC	95°C for 2 s 60°C for 6 s 72°C for 10 s	219

SYBR Green I dye and dNTP mix). After an initial Taq activation at 95°C for 10 min, LightCycler PCR was performed using 40 cycles with the cycling conditions described in Table 1. In order to verify the purity of the products, a melting curve was produced after each run by increasing the temperature of the reaction mixtures up to 95°C, by 0.1°C/s, starting at 55°C for 10 s. Values for the threshold (Ct) were determined using the LightCycler software [33].

Relative quantification of gene expression was calculated based on efficiency and the crossing point deviation of an unknown sample versus a control and expressed in comparison to a reference gene (18S) [34].

2.5. Statistical analysis

All data are expressed as the mean±S.E.M. One-way ANOVA was used to assess differences between depots under control conditions and between control and the three fasted groups in each depot. Least significant difference (LSD) test was used for post hoc comparisons. Student's *t* test was used for single differences of the refed group versus the 8-h fasting group and versus the control group, and repeated-measures Student's *t* test was used to assess differences between body weight before fasting and at sacrifice. Two-way ANOVA was used to determine the existence of interactive effects of depot and feeding condition. The analyses were performed with SPSS for Windows (SPSS, Chicago, IL). Threshold of significance was defined at $P<.05$.

The authors certify that all applicable institutional and governmental regulations concerning the ethical use of animals were followed during this research. The animal protocol followed in this study was reviewed and approved by the Bioethical Committee of the University, following its guidelines for the use and care of laboratory animals.

3. Results

3.1. Body weight, adipose tissue weights and blood parameters

Initial and final body weight, the weight of the different adipose tissue depots (retroperitoneal, mesenteric and inguinal) and blood parameters are summarized in Table 2. Body weight of animals decreased significantly from 4 h of fasting onwards, with respect to their initial body weight before fasting ($P<.05$, Student's t test), as previously described [31]. The weights of the different adipose tissue depots under fasting conditions were not significantly different to those of the animals under ad libitum feeding conditions, although a tendency to decrease was observed after 24 h of fasting. This tendency was more marked for the retroperitoneal depot (decrease of 13.9%), followed by the mesenteric depot (11.0%) and lastly by the inguinal depot (7.8%). Three hours of refeeding after 8 h of fasting resulted in a significant increase in body weight ($P<.05$, Student's t test) but did not significantly alter the weight of fat depots.

Concerning circulating blood parameters, there was a normal physiological response to fasting, as previously described [31]. Particularly, there was a rapid decrease in circulating insulin levels, already significant after 4 h of fasting, which continued decreasing at 24 h of fasting ($P<.05$, one-way ANOVA). Circulating levels of NEFA increased significantly also within 4 h after food deprivation, peaking at 8 h, thus indicating that NEFA are very sensitive to nutritional variations; blood glucose concentration was found to be significantly decreased from 8 h of fasting onwards and β-hydroxybutyrate increased from 8 h of fasting ($P<.05$, one-way ANOVA). Three hours of refeeding after 8 h of fasting reversed the effect of fasting on these parameters ($P<.05$, Student's t test).

3.2. Effect of fasting and refeeding in the expression of lipogenesis-related genes in different fat depots

The expression of lipogenesis-related genes in the adipose tissue decreased under fasting conditions but in a depot-specific manner. A faster, more generalized response was found in the retroperitoneal depot. Concerning transcription factors involved in lipogenesis (Table 3), a significant decrease in the mRNA expression levels of PPARγ2 was already found after 4 h of fasting and of SREBP1c after 8 h of fasting in the retroperitoneal depot. SREBP1c mRNA levels also decreased in the inguinal depot at 8 h of fasting, while no significant changes were found in the mesenteric depot. Three hours of refeeding after 8 h of fasting reversed the effect of fasting on PPARγ2 and SREBP1c mRNA expression levels in the retroperitoneal depot and also increased PPARγ2 mRNA expression (which was not affected by fasting) in the inguinal depot, without producing changes in the mesenteric depot.

The expression of genes encoding other proteins involved in lipogenesis was also affected by fasting (Table 4). In the retroperitoneal depot, FAS, GPAT and GLUT4 mRNA levels were found to be significantly lower after 8 h of fasting with respect to the expression levels under ad libitum feeding conditions ($P<.05$, one-way ANOVA). ACC1 mRNA levels after 8 and 24 h of fasting were significantly lower with respect to levels after 4 h of fasting ($P<.05$, one-way ANOVA) but not with respect to control levels. In the mesenteric depot, only GLUT4 mRNA levels were found to be significantly lower after 8 h of fasting and FAS mRNA levels after 24 h of fasting, with respect to the expression levels under ad libitum feeding conditions ($P<.05$, one-way ANOVA). However, in this depot, a peak in the expression of some genes related with lipogenesis appeared to occur after 4 h of fasting; in fact, FAS and GPAT mRNA levels after 8 h of fasting — although not different to those under ad libitum feeding conditions — were significantly lower compared with those after 4 h of fasting. In the inguinal depot, ACC1, FAS and LPL mRNA expression levels were found to be significantly lower after 8 h of fasting with respect to the expression levels under ad libitum feeding conditions ($P<.05$, one-way ANOVA), but no significant changes were found in the expression levels of GPAT and GLUT4

Table 2
Weight-related and serum biochemical parameters

	Ad libitum	4 h of fasting	8 h of fasting	24 h of fasting	3 h of refeeding
Initial body weight (g)	366±3	366±7	368±2	367±4	366±3
Body weight at sacrifice (g)	366±3 (a)	359±7 (a,b)**	349±3 (b)**	335±4 (c)**	358±3*
rWAT (g)	3.31±0.35	3.31±0.32	4.13±0.90	2.85±0.40 (↓13.9% vs. Ad Lib)	3.32±0.44
mWAT (g)	2.26±0.17	2.40±0.12	2.40±0.26	2.01±0.13 (↓11.0% vs. Ad Lib)	2.57±0.33
iWAT (g)	3.62±0.20	3.46±0.12	3.98±0.29	3.33±0.15 (↓7.8% vs. Ad Lib)	3.57±0.16
Glucose (mg/dl)	104±3 (a)	98±2 (a)	88±3 (b)	79±3 (c)	109±3*
Insulin (μg/L)	1.30±0.15 (a)	0.637±0.066 (b)	0.479±0.083 (b,c)	0.340±0.019 (c)	1.03±0.18*
NEFA (mol/L)	413±69 (a)	670±49 (b,c)	714±47 (c)	546±40 (a,b)	240±45*
β-Hydroxybutyrate (μg/ml)	28.7±9.1 (a)	40.7±7.1 (a)	86.8±11.2 (b)	153±19 (c)	29.6±8.9*

Initial body weight (before fasting) and body weight at sacrifice; weights of retroperitoneal, mesenteric and inguinal white adipose tissue (rWAT, mWAT and iWAT, respectively); and blood glucose concentration and plasma concentration of insulin, NEFA and β-hydroxybutyrate in rats under different feeding conditions: ad libitum feeding state; after 4, 8 or 24 h of fasting; and after 3 h of refeeding following 8 h of fasting. Weights are expressed in grams. The percentage decrease in the weight of fat depots after 24 h of fasting is indicated. Data are means±S.E.M. (n=6–8). a≠b≠c ($P<.05$, one-way ANOVA and LSD post hoc test).

* Three hours of refeeding versus 8 h of fasting ($P<.05$, Student's t test).

** Body weight at sacrifice versus initial body weight ($P<.05$, repeated-measures Student's t test).

Table 3
Expression of the lipogenic transcription factors PPARγ2 and SREBP1c in the retroperitoneal, mesenteric and inguinal white adipose tissue (rWAT, mWAT and iWAT, respectively) under different feeding conditions: ad libitum feeding state; after 4, 8 or 24 h of fasting; and after 3 h of refeeding following 8 h of fasting

	Ad libitum	4 h of fasting	8 h of fasting	24 h of fasting	3 h of refeeding
rWAT					
PPARγ2	100±12 (a)	67.8±10.8 (b)	53.8±11.1 (b)	62.0±6.3 (b)	127±20*
SREBP1c	100±7 (a)	87.3±12.4 (a)	55.8±5.7 (b)	55.6±8.9 (b)	107±18*
mWAT					
PPARγ2	100±14	89.9±16.2	68.2±8.3	66.8±16.8	66.4±7.9
SREBP1c	100±12	141±35	65.4±10.1	64.3±12.8	57.5±1.8
iWAT					
PPARγ2	100±10	76.8±20.3	82.5±16.4	127±37	220±55*
SREBP1c	100±15 (a)	65.0±13.8 (a,b)	49.0±11.6 (b)	43.4±11.2 (b)	68.7±20.4

mRNA levels were measured by real-time PCR and expressed as a percentage of the mean value of animals under ad libitum feeding conditions. Data are means±S.E.M. (n=6–8). a≠b (P<.05, one-way ANOVA and LSD post hoc test).

* Three hours of refeeding versus 8 h of fasting (P<.05, Student's t test).

as an effect of fasting. Of note, the effect of fasting on GLUT4 and LPL expression levels was different in the depots studied (interaction between the effect of fasting and the tissue, P<.05, two-way ANOVA); particularly, the pattern of response to fasting of both genes in the inguinal depot was significantly different to that of the retroperitoneal and mesenteric depots (P<.05, two-way ANOVA).

Three hours of refeeding after 8 h of fasting was ineffective in reverting the effect of fasting on ACC1, FAS, GPAT, GLUT4 and LPL mRNA expression levels in any depot, and even FAS (in both the retroperitoneal and inguinal depots) and GLUT4 (in the retroperitoneal depot) continued to decrease with the mRNA levels becoming significantly lower than those found after 8 h of fasting (P<.05, Student's t test). Moreover, mRNA expression levels of LPL in the mesenteric depot, which remained unaltered under fasting conditions, decreased as an effect of refeeding.

3.3. Effect of fasting and refeeding on the expression of lipolytic and fatty acid oxidation-related genes in different fat depots

The expression of the studied genes related with lipid mobilization and fatty acid oxidation was only slightly affected at the transcriptional level by food deprivation, although differences between depots were also found. Concerning the expression of genes encoding for enzymes involved in lipid mobilization (Table 5), HSL mRNA expression was not significantly affected by the assessed short- and medium-time periods of food deprivation in any of the studied depots, while ATGL expression increased

Table 4
Expression of genes encoding proteins involved in lipogenesis in the retroperitoneal, mesenteric and inguinal white adipose tissue (rWAT, mWAT and iWAT, respectively) under different feeding conditions: ad libitum feeding state; after 4, 8 or 24 h of fasting; and after 3 h of refeeding following 8 h of fasting

	Ad libitum	4 h of fasting	8 h of fasting	24 h of fasting	3 h of refeeding
rWAT					
ACC1	100±13 (a,b)	126±29 (a)	66.8±10.2 (b)	58.7±9.0 (b)	47.7±6.8
FAS	100±13 (a)	135±27 (a)	51.2±9.6 (b)	27.5±9.8 (b)	23.4±4.0*
GPAT	100±18 (a)	88.2±13.0 (a)	43.8±5.5 (b)	44.3±6.5 (b)	44.8±5.2
*GLUT4***	100±19(a)	128±18 (a)	50.1±6.0 (b)	23.3±7.2 (b)	28.8±7.2*
*LPL***	100±12	90.8±7.3	101±8	93.0±11.1	91.0±15.0
mWAT					
ACC1	100±30	125±46	38.7±8.8	57.1±22.4	26.5±4.1
FAS	100±29 (a,b)	212±47 (a)	47.3±8.9 (b,c)	23.9±9.7 (c)	36.0±11.8
GPAT	100±19 (a,b)	143±32 (a)	61.7±11.2 (b)	59.3±13.9 (b)	51.5±8.3
*GLUT4***	100±19 (a)	177±55 (a)	40.3±12.1 (b)	21.3±8.1 (b)	31.0±13.0
*LPL***	100±14	193±30	112±18	118±37	57.4±7.1*
iWAT					
ACC1	100±17(a,c)	109±20 (a)	37.4±6.7 (b)	51.2±4.4 (b,c)	66.1±13.9
FAS	100±18(a)	95.7±11.2 (a)	31.5±10.5 (b)	15.8±10.9 (b)	2.87±0.86*
GPAT	100±24	72.0±13.0	41.9±9.7	59.9±12.1	74.7±13.0
*GLUT4***	100±15	46.0±7.3	71.6±18.8	76.5±21.8	103±21
*LPL***	100±12(a)	81.7±14.5 (a,b)	50.2±6.1 (b)	61.8±13.4 (b)	83.8±16.5

mRNA levels were measured by real-time PCR and expressed as a percentage of the mean value of animals under ad libitum feeding conditions. Data are means±S.E.M. (n=6–8). a≠b≠c (P<.05, one-way ANOVA and LSD post hoc test).

* Three hours of refeeding versus 8 h of fasting (P<.05, Student's t test).

** Interaction between the effect of fasting and the adipose tissue depot, rWAT and mWAT≠iWAT (P<.05, two-way ANOVA).

Table 5
Expression of the enzymes involved in lipolysis: HSL and ATGL, in the retroperitoneal, mesenteric and inguinal white adipose tissue (rWAT, mWAT and iWAT, respectively) under different feeding conditions: ad libitum feeding state; after 4, 8 or 24 h of fasting; and after 3 h of refeeding following 8 h of fasting

	Ad libitum	4 h of fasting	8 h of fasting	24 h of fasting	3 h of refeeding
rWAT					
HSL	100±8	105±8	106±12	107±16	126±19
ATGL	100±12 (a)	114±10 (a)	121±13 (a)	168±23 (b)	182±46
mWAT					
HSL	100±18	164±30	125±19	121±28	63.1±7.4*
ATGL	100±18	145±34	141±31	207±55	77.5±9.2
iWAT					
HSL	100±25	57.1±15.1	63.8±16.1	105±48	145±46
ATGL	100±24	45.8±9.6	71.4±15.0	139±70	213±85

mRNA levels were measured by real-time PCR and expressed as a percentage of the mean value of animals under ad libitum feeding conditions. Data are means±S.E.M. (n=6–8). a≠b (P<.05, one-way ANOVA and LSD post hoc test).

* Three hours of refeeding versus 8 h of fasting (P<.05, Student's t test).

significantly after 24 h of fasting in the retroperitoneal depot (P<.05 one-way ANOVA). No significant changes were found in the other depots studied, although a tendency to increase was observed in the mesenteric depot. Three hours of refeeding after 8 h of fasting did not significantly affect mRNA expression levels of the studied genes related with lipid mobilization, with the exception of HSL, whose mRNA expression levels were found to be decreased, but only in the mesenteric depot (P<.05, Student's t test).

The expression of genes encoding proteins related with fatty acid oxidation, particularly the transcription factor PPARα and the enzymes CPT1 and ACOX1, was only slightly affected by fasting, but also in a depot-specific manner (Table 6). PPARα mRNA levels were not significantly altered under fasting conditions in any of the studied depots. CPT1 mRNA levels were found to be significantly higher after 24 h of fasting in the retroperitoneal depot, whereas they decreased in the inguinal depot; no significant changes were found in the mesenteric depot, although a tendency to increase was appreciated, particularly after 24 h of fasting. Of note, the effect of fasting on CPT1 expression levels was different in the depots studied (interaction between the effect of fasting and the tissue, P<.05, two-way ANOVA); particularly, the pattern of response to fasting of this gene in the inguinal depot was significantly different to that of the retroperitoneal and mesenteric depots (P<.05, two-way ANOVA). mRNA levels of ACOX1 remained unchanged under fasting conditions in the different depots studied. Three hours of refeeding after 8 h of fasting did not significantly affect mRNA expression levels of the studied genes related with fatty acid oxidation.

4. Discussion

The main function of adipose tissue is to store energy in the form of triacylglycerides and to mobilize this fuel when needed. Fat storage and fat mobilization during normal daily life are controlled by coordinated regulation of a number of enzymatic processes, which are regulated at different levels [35]. However, adipose tissue is distributed in different anatomical sites, both subcutaneously and internally, which have different biological functions and metabolic activities [1]; hence, the mechanisms of fat accumulation and mobilization may differ significantly between depots. This study was undertaken to characterize the time-course changes in the expression of genes encoding for key proteins involved in the lipolytic and lipogenic pathways in adipose tissues from different anatomical localization in an experimental model of short- and medium-term periods of fasting and refeeding, a situation that may mimic the habitual food intake/fasting pattern in humans [2].

Table 6
Expression of genes encoding proteins involved in fatty acid oxidation in the retroperitoneal, mesenteric and inguinal white adipose tissue depots (rWAT, mWAT and iWAT, respectively) under different feeding conditions: ad libitum feeding state; after 4, 8 or 24 h of fasting; and after 3 h of refeeding following 8 h of fasting

	Ad libitum	4 h of fasting	8 h of fasting	24 h of fasting	3 h of refeeding
rWAT					
PPARα	100±12	107±15	71.5±8.9	65.0±3.5	106±27
*CPT1**	100±18 (a)	183±23 (a,b)	171±18 (a,b)	250±59 (b)	215±5
ACOX1	100±34	149±43	167±21	98.1±36.3	158±17
mWAT					
PPARα	100±16	115±20	88.2±13.8	76.6±13.9	80.2±5.3
*CPT1**	100±11	97.9±18.0	150±33	178±34	114 ±30
ACOX1	100±9	121±27	62.0±19.3	79.9±27.0	65.3±18.4
iWAT					
PPARα	100±20	82.1±13.3	64.3±5.4	43.6±5.8	85.6±35.6
*CPT1**	100±20 (a,b)	117±14 (a)	73.7±17.1 (a,b)	46.6±16.9 (b)	58.7±20.9
ACOX1	100±16	99.4±8.2	63.7±17.6	74.4±23.0	103±17

mRNA levels were measured by real-time PCR and expressed as a percentage of the mean value of animals under ad libitum feeding conditions. Data are means±S.E.M. (n=6–8). a≠b (P<.05, one-way ANOVA and LSD post hoc test).

* Interaction between the effect of fasting and the adipose tissue depot, rWAT and mWAT≠iWAT (P<.05, two-way ANOVA).

According to previously published results [31], the increase in circulating NEFA levels (already occurring after 4 h of fasting) and that of β-hydroxybutyrate (after 8 h of fasting) were very fast events occurring after food deprivation, indicative of the fact that a main source of energy for the organism at this time derives from adipose tissue lipid mobilization and its hepatic utilization [31], in addition to the hepatic glycogen stores; however, we show here that the adaptation to food deprivation at the transcriptional level differs considerably between depots considering both fat mobilization and also the inhibition of lipogenesis, which is also a feature of a situation of negative energy balance and completes the picture of fat balance.

PPARγ and SREBP1c are both important transcription factors with a relevant role in lipogenesis. In the retroperitoneal depot, we found that the decrease in PPARγ2 expression levels was a rapid response to fasting, already occurring after only 4 h of fasting. However, no significant changes concerning PPARγ2 expression were found in the other depots studied as an effect of fasting. PPARγ expression is known to be regulated by insulin [36]. Thus, the decrease in insulin levels, which occurred after 4 h of fasting, contributes to explain the decrease in the mRNA expression of PPARγ2 in the retroperitoneal depot. In fact, we found a positive correlation between plasma insulin levels and PPARγ2 mRNA levels (Pearson's correlation coefficient r=.442, P<.05) but only in the retroperitoneal depot and not in the other adipose tissue depots. However, variations in plasma insulin concentrations do not account for the lack of a significant response occurring in the other depots, particularly in the inguinal depot. An explanation could lie in the site-specific regulation of proteins involved in insulin signal transduction as well as in the different depot-related responsiveness to catecholamines [23,37–39].

The decrease in PPARγ2 expression in the retroperitoneal depot was followed by a decrease in GLUT4 expression, occurring after 8 h of fasting, in accordance with the stimulatory role of PPARγ on GLUT4 expression [11]. A decrease in GLUT4 expression was also found in the mesenteric depot, associated to a tendency to lower expression levels of PPARγ2, but it remained unchanged in the inguinal depot. Previous studies in rats have also demonstrated that GLUT4 expression in the adipose tissue is down-regulated under fasting conditions and that insulin is a major factor regulating its mRNA levels in this tissue [40]. Of interest, we show here depot-specific differences in the time-course adaptation to food deprivation concerning the expression of this gene, with a faster response in internal depots compared with the subcutaneous depot.

In addition to GLUT4, PPARγ has been shown to stimulate the expression of LPL [1,8], whose activity is an important determinant of triglyceride storage in the adipocyte [41]. LPL catalyzes the hydrolysis of circulating triglycerides to free fatty acids, which can then be reesterified and stored in the adipocyte. Regional variations in adipose tissue LPL activity have been demonstrated, and these differences closely parallel regional variations in fat cell size [42,43]. In addition, it is known that LPL activity changes during the day according to the nutritional state [44]. Its regulation can involve several mechanisms, most likely dependent on the timescale of the required response and the effector involved [45]. During short-term fasting, LPL activity in rat adipose tissue has been shown to decrease without corresponding changes in the levels of mRNA and protein mass, due to a reduced specific activity of the enzyme (activity/protein mass ratio) [46]. However, we show here differences in the regulation of LPL mRNA expression depending on the anatomical localization. Whereas no significant changes were found as an effect of fasting in LPL mRNA levels in internal adipose tissue depots, a significant decrease was found in the inguinal depot after 8 h of fasting. Thus, the expression of genes encoding for proteins involved in the supply of glucose (GLUT4) and fatty acids from lipoproteins (LPL) to the adipose tissue in the different depots was differentially affected by food deprivation. These changes at the transcriptional level are not necessarily translated into changes in the protein content and or activity, but if so, this would mean that under a situation of food deprivation, the retroperitoneal and the mesenteric depots would maintain fatty acid uptake capacity from lipoproteins, whereas decreased GLUT4 expression would save glucose to be used by other tissues. However, in the inguinal depot, the decrease in LPL expression levels under fasting conditions could indicate lower fatty acid uptake capacity from lipoproteins, while the glucose uptake capacity through the GLUT4 receptor would remain unaltered.

Insulin is an important regulator of the expression of SREBP1c, which therefore mediates insulin effects on lipogenesis in adipocytes [47]. In the retroperitoneal and inguinal depots, a decrease in the expression of SREBP1c was found after 8 h of fasting and was accompanied by a significant decrease in FAS expression, although the response of the mesenteric depot to fasting concerning SREBP1c expression was not statistically significant and a significant decrease in FAS expression was only found after 24 h of fasting, showing that the mesenteric depot is less responsive to the fasting-induced inhibition of the expression of lipogenic-related genes. In addition to changes in FAS expression, the expression of other genes encoding for enzymes involved in lipogenesis, such as ACC1 and GPAT, also showed a tendency to decrease after 8 h of fasting in the three depots studied but in a depot-specific manner: the decrease in GPAT expression was only statistically significant in the retroperitoneal depot and that of ACC1 was only statistically significant in the inguinal depot.

The mobilization of stored triacylglycerides in the adipose tissue seems to be largely initiated by ATGL, afterwards followed by HSL, the latter acting mainly on diacylglycerides [48]; thus, ATGL could be an important key enzyme in triglyceride mobilization, acting upstream to HSL. This idea is reinforced by the results shown here, since

HSL mRNA levels remained unaltered upon fasting, while ATGL mRNA levels were increased after 24 h of fasting in the retroperitoneal depot and showed the same (nonsignificant) tendency in the mesenteric one but not in the inguinal depot. In mice, ATGL mRNA levels have been found to be transiently elevated after 12 and 24 h of fasting [6], and in vitro studies have demonstrated that ATGL expression is up-regulated by dexamethasone, but not by cAMP, suggesting that glucocorticoids could mediate the increase in ATGL mRNA during fasting [6]. Of interest, although mean values of the weights of the adipose tissue depots studied under fasting conditions were not significantly different compared with values under ad libitum feeding conditions, a greater tendency to decrease was found after 24 h of fasting in the retroperitoneal depot, followed by the mesenteric depot and finally by the inguinal depot. This pattern agrees with the changes occurring in the expression of ATGL in the different depots as an effect of fasting. Thus, our results support the role of ATGL in the adaptive response of the adipose tissue, particularly in the retroperitoneal depot and also in the mesenteric depot, to face a situation of negative energy balance such as fasting. This enzyme seems to be, at the transcriptional level, more sensitive to this metabolic challenge than HSL, although neither was changed at the very early stages of fasting.

On the other hand, it must also be taken into account that the lack of changes in HSL mRNA levels shown here does not preclude the existence of changes in HSL expression for a longer fasting duration. In fact, a lack of response at the translational level in HSL has been previously described after 1 day of fasting, although a significant increase in retroperitoneal adipose tissue, but not in subcutaneous adipose tissue, has been described after 5 days of fasting [39]. Moreover, the increase in circulating NEFA was already found after 4 h of fasting. Thus, an up-regulation of the enzyme activity, occurring previously to changes at the translational level, together with reduced triacylglyceride synthesis and storage, is most likely to be involved in the rapid increase in circulating NEFA under fasting conditions. Increased triacylglyceride mobilization during short-term fasting appears to involve posttranslational control of HSL; it is during long-term (3–5 days) fasting when HSL activity appears to be regulated by pretranslational mechanisms [49].

Increased expression of ATGL in the retroperitoneal depot under fasting conditions was also accompanied by increased capacity of mitochondrial oxidation of fatty acids, since higher CPT1 mRNA levels were also found after 24 h of fasting; however, the expression of CPT1 mRNA levels did not change significantly in the mesenteric depot and even decreased in the subcutaneous depot. Of note, in the subcutaneous depot, the decrease in CPT1 mRNA levels after 24 h of fasting appears to be related to the aforementioned decrease in LPL mRNA levels already occurring after 8 h of fasting, suggesting that under a situation of food deprivation, this depot could have lower capacity to oxidize fatty acids.

Refeeding after a period of fasting also involves a highly coordinated adaptation of the expression of genes encoding key metabolic enzymes in the adipose tissue, which is orchestrated by hormones and nutrients. The timing of these adaptations could also be expected to be both region dependent and gene dependent. Here, we have seen that the retroperitoneal depot also appears as the fat depot with the fastest metabolic response to this challenge, as also seen with the adaptations under food deprivation. In particular, the decrease in PPARγ2 and SREBP1c mRNA levels observed after 4 and 8 h of fasting, respectively, was reverted by only 3 h of refeeding. A significant increase in PPARγ2 expression was also found in the inguinal depot, while the mesenteric depot — which did not experience significant changes after 8 h of fasting concerning the expression of these transcription factors — remained unaffected by this period of refeeding. Thus, PPARγ2 in both the retroperitoneal and inguinal depots and SREBP1c in the retroperitoneal depot are very sensitive to the nutritional status. These changes are associated with the increase in blood insulin levels, which is a very fast event occurring after refeeding.

However, in the retroperitoneal tissue, this period of refeeding was not enough to recover the expression levels of genes encoding proteins involved in lipogenesis (ACC1, FAS, GPAT) and even the expression levels of FAS continued to decrease after refeeding as also happened with GLUT4 expression. A decrease in FAS expression after refeeding was also found in the inguinal depot, illustrative of the slow response of this gene to feeding conditions. LPL mRNA expression levels in the mesenteric depot experienced a significant decrease after 3 h of refeeding. It should be noted that the expression of this gene was not significantly affected by fasting in this fat depot; thus, although we have not determined changes in LPL activity, changes in mRNA levels would show a regional adaptive response to preserve fuel to be used by other tissues instead of being stored as triacylglycerides in this tissue under this short-term period of refeeding.

The expression of genes related with lipid mobilization and fatty acid oxidation, whose expression levels were not altered after 8 h of fasting in any of the adipose tissue studied, also remained mostly unaltered after refeeding. However, HSL mRNA levels decreased in the mesenteric depot after 3 h of refeeding, compared with their expression levels after 8 h of fasting. This decrease suggests the existence of a different regulatory mechanism operating in this depot under fasting/refeeding conditions to regulate triacylglyceride mobilization: while translational or post-translational mechanisms appear to regulate HSL activity under fasting conditions, transcriptional mechanisms or changes in HSL mRNA turnover would seem to be involved in the response of this depot to refeeding. A decrease in circulating NEFA was already found after 3 h of refeeding, which would indicate that a decrease in the activity of HSL and/or ATGL must be a very rapid mechanism operating in response to short-term period of refeeding after fasting.

All in all, although we have not analyzed changes in protein levels, these results based on mRNA expression provide molecular bases that can account, at least in part, for the preferential fasting-induced mobilization of triacylglycerides from the retroperitoneal adipose tissue depot, compared with the mesenteric and the inguinal depots. A sequence of events and the representative picture of metabolic adaptations in the subcutaneous and internal fat depots can be deduced. The faster induction of lipogenic transcription factors PPARγ2 and SREBP1c in the retroperitoneal depot compared with the others after refeeding is also in accordance with the concept that the retroperitoneal depot has a higher metabolic activity compared with the other depots and particularly with the subcutaneous one, as previously pointed out [26]. Although we have not determined the molecular mechanisms mediating the metabolic differences observed between depots, the results obtained suggest that the retroperitoneal depot could be more responsive to the action of insulin and catecholamines than the other depots. The data presented here extend our understanding of the rationale for gene expression in fat from different anatomical regions and provide further proof that the mechanisms of fat accumulation and mobilization differ significantly between depots.

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