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ORIGINAL CONTRIBUTION

Site-related white adipose tissue lipid-handling response to oleoyl-estrone treatment in overweight male rats

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Abstract
Background Oleoyl-estrone (OE) decreases energy intake while maintaining glucose homeostasis, and energy expenditure at the expense of body fat. White adipose tissue (WAT) depots behave differently under starvation, postprandial state and pharmacologically induced lipolysis.
Aim of the study To understand the mechanism of massive lipid loss from WAT elicited by OE treatment.
Methods We used overweight male rats. Rats receiving OE (10 nmol/g) gavages were compared with controls and a pair-fed group. Whole fat pads from the mesenteric, retroperitoneal, epididymal and inguinal subcutaneous sites were excised and analyzed for lipid, DNA, mRNA and the expression of lipogenic, fatty acid transporters and lipase genes.
Results In OE and pair-fed rats, WAT weights decreased, with the limited loss of cells. Patterns of gene expression in most WAT sites were similar for OE and PF, suggesting a shared mechanism of fat mobilization, but in mesenteric WAT, PF increased lipogenic and fatty acid transporter gene expressions. However, OE inhibited lipogenic expressions more deeply than PF.
Conclusions White adipose tissue sites showed different expression patterns, hinting at relatively specialized functions in fat storage; thus, single site analyses cannot be extrapolated to whole WAT. Differences between mesenteric and the other sites suggest that 'visceral fat' should be reserved for this site only, and not applied to other abdominal fat depots (epididymal, retroperitoneal).

Keywords White adipose tissue · Obesity · Oleoyl-estrone · Visceral fat · Lipogenesis

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Introduction

Obesity, a key constituent of the metabolic syndrome, is a direct correlate of the mass and distribution of white adipose tissue (WAT), and obesity treatments are focused on the elimination of these fat depots. WAT stores most of the body triacylglycerol reserves, and this storage function is yet considered to be its main function despite growing awareness of its many additional functions as stem cell repository [27], endocrine [36], paracrine [17], and immune/defense [22] site. WAT also influences, through paracrine secretion, the response of neighboring vessels and organs [15], and acts as insulating, filling, protecting or spacing material. Its site-dispersed nature and the multiple functions carried out by WAT agree with a logical specialization of different sites in functions additional to

energy storage, which translates into a variable structuring of cell types, sizes and expression patterns. The existence of metabolically differentiated 'visceral' and 'subcutaneous' fat depots has been repeatedly associated with different forms and intensities of pathological situations such as the metabolic syndrome [6] and, consequently, there is a higher cardiovascular risk for increased visceral versus subcutaneous fat carriers [25]. However, this gross distinction is often insufficient, since the term 'visceral' is imprecise and comprises a variety of fat pads with different and distinct anatomical locations inside the visceral cavity, with different irrigation schemes that largely determine their different physiological function.

There is a number of studies on the composition and metabolic orientation of the WAT at different sites of the mammal [5, 21], especially oriented to their different ability to express or release cytokines [30, 44], other hormones [12] or their potential response to metabolic regulation agents [31, 45]. The direct analysis of their handling of fat has been sparsely analyzed in relation to site distribution.

Oleoyl-estrone is a powerful slimming agent [41] that induces an energy imbalance by decreasing food intake and maintaining energy expenditure [40]. The action of OE results in the maintenance of body protein [13, 41] and glycemia as well as liver glycogen [38]. However, muscle utilization of lipid in obese rats is enhanced in parallel to the normalization of circulating lipids and the wasting of overall body fat [4]. The administration of OE induces the fast loss of body fat with little change on other metabolic parameters (e.g. protein turnover/nitrogen balance, glycemia) [13, 40, 41].

A key objective of this study was to determine how OE exerts this marked reduction in adipose tissue lipid. However, in order to differentiate the effects of OE from those elicited by reduced food intake, a group of animals pair-fed with respect to those receiving OE was included. This study allowed us to establish basal and OE-induced differences in the main agents regulating triacylglycerol metabolism through gene expression.

An additional objective of the study was to determine whether OE affected differently the main WAT sites and the eventual attribution of these effects to its hormonal signaling [1] other than those simply derived from limited access to food.

The overweight (and otherwise normal) rat model [10] allows for a fair anatomical distinction of a number of WAT sites, yielding sufficient material for analysis and adequate 'slimming times' for investigative consistency. Thus, we used medium-sized overweight male rats to ascertain whether the loss of fat caused similar decreases in the most commonly studied WAT sites, and, especially, whether the abdominal (i.e. 'visceral') adipose tissues behaved in a similar way as to made any of them representative of WAT metabolic changes as assumed in many studies that distinguish only 'visceral' and 'subcutaneous' WAT.

Methods

Animals and sample preparation

Adult male Wistar rats were made overweight by a limited period of cafeteria diet feeding, as previously described [10]. The rats, initially weighing 355 ± 5 g, were kept under standard conditions of housing and feeding [10]. Three groups of eight rats each were randomly selected: controls, OE and 'pair-fed' (PF). All animals received every day an oral gavage of 0.2 mL of sunflower oil (7 kJ), which was supplemented in the OE group with 10 nmol/g oleoyl-estrone (OED, Barcelona, Spain). The controls and OE group had free access to pellet food (maintenance chow, Panlab, Barcelona, Spain), and the PF rats were allowed to eat only every day the mean food consumption on the matching day of the OE group; all rats had water available ad libitum. On day 10, the rats were killed by decapitation. The following WAT sites were isolated and completely dissected: intestine-related mesenteric WAT [MAT], perigonadal (epididymal) [EAT], retroperitoneal cordons [RPAT] and the subcutaneous inguinal [SCAT] fat pads. The samples were blotted and carefully cleaned of extraneous material (epididymis, pancreas, dermis), weighed, frozen in liquid nitrogen and kept at −80° C.

The animals were kept, handled and killed following the specific procedures approved by the University of Barcelona Animal Welfare and Ethics Committee, in full conformity with the norms and proceedings set forth by the European Union and the Governments of Spain and Catalonia.

Nucleic acid analyses, cellularity

Tissue samples were used for the estimation of total DNA, using a standard fluorimetric method with 3,5-diaminobenzoic acid (Sigma, St Louis MO, USA) and bovine DNA (Sigma) as standard [46]. Tissue DNA content allowed the calculation of the number of cells per gram of tissue and in the whole tissues sampled, based on the assumption that the DNA content per cell is constant in mammals; here, we used the genomic DNA size data [26] for somatic rat cells (5.6 pg/cell). Mean cell mass was estimated from the number of cells and the weight of the organ.

Total tissue RNA was extracted using the Tripure reagent (Roche Applied Science, Indianapolis, IN, USA), and were quantified in a ND-100 spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA). Tissue total mRNA was determined using the poly-(A) mRNA detection system kit (Promega).

Semiquantitative RT-PCR analysis of protein expression

RNA samples were reverse transcribed using the MMLV reverse transcriptase (Promega, Madison, WI USA) and oligo-dT primers.

Real-time PCR (RT-PCR) amplification was carried out using 0.010 mL amplification mixtures containing Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA), equivalent to 8 ng of reverse-transcribed RNA and 300 nM primers. Reactions were run on an ABI PRISM 7900 HT detection system (Applied Biosystems) using a fluorescent threshold manually set to OD 0.500 for all runs.

We have grouped the studied genes in three groups: (1) lipogenic (citrate: ATP lyase, acetyl-CoA carboxylase, fatty acid synthase and glycerol-3P acyl-transferase; (2) proteins binding/translocating or transporting fatty acids (fatty acid transporter protein 1, fatty acid translocase, carnitine-palmitoleoyl transferase, and fatty acid-binding protein 4); and (3) lipases (lipoprotein lipase, hormone-sensitive lipase, adiponutrin, and adipose triacylglycerol lipase). The list of primers used is given in Supplemental Table 1.

A semiquantitative approach for the ultimate estimation of the number of copies of each expressed gene mRNAs per tissue weight was used as previously described [34]. In any case, cyclophilin was used as charge control gene in all samples.

Statistical comparison between the groups was established using the unpaired Student's t test and one-way ANOVA tests, with a limit of significance of $P < 0.05$.

Results

Body weight and food intake

During the 10-day period studied, control rats gained 9.1 ± 1.4 g (i.e. 2.6% of initial body weight), while OE rats lost 34.9 ± 2.3 g (9.9%), and the pair-fed animals lost 29.8 ± 2.4 g (8.5%), the differences versus controls being significant for both OE and PF rats. Mean daily food consumption was 18.0 ± 0.2 g (i.e. 257 kJ) for controls, and 10.4 ± 0.3 g (187 kJ) for both OE and PF rats (i.e. a mean 58% of controls), the differences being statistically significant.

Nucleic acid content and cellularity

Table 1 shows the weight and cellularity of the four WAT sites studied; in the PF group, the loss of weight for the WAT sites was: RPAT 45%, EAT 41%, MAT 37% and SCAT 33%; OE rats lost slightly less: RPAT 45%, MAT 36%, and EAT or SCAT 30%. In all cases, the differences were significant versus controls but not between OE and PF groups.

The mean cell weight was higher for RPAT, followed by EAT and SCAT; MAT showing the lowest mean size, less than half the RPAT mean cell weight. However, and despite the loss of weight, the number of cells contained in WAT pads changed little, with only significant losses for PF EAT, and PF and OE RPAT. As a consequence, the mean cell size decreased significantly in all sites (PF and OE alike) versus controls, except for RPAT of OE-treated rats.

The proportion of mRNA with respect to total RNA showed little overall differences in controls (2.8–3.5%); the range for PF was 2.7–4.0, and 2.7–4.6% for OE rats. However, the ratio of total RNA to DNA increased in all sites in OE and PF rats, with the exception of unchanged ratio in the OE EAT and decreased ratios in SCAT samples.

Gene expression analysis

Table 2 presents the lipid-related gene expressions in the four WAT sites of control rats. Arbitrarily using SCAT as reference, values for lipogenic enzyme expressions tended to be higher in RPAT (and, to a lesser extent, MAT) when expressed per unit of tissue weight, while EAT tended to be lower. The pattern for fatty acid transporters (i.e. fatty acid transport protein 1, the translocase and carnitine-palmitoleoyl transferase genes) were similar in SCAT, RPAT and MAT, but lower in EAT. Fatty acid-binding protein gene showed the highest abundance of specific mRNA in RPAT and lowest in EAT. The four lipase genes studied showed different patterns, with closely similar expressions for adiponutrin gene in all sites, lowest lipoprotein lipase and hormone-sensitive lipase gene expressions in EAT, and highest adipose triacylglycerol lipase gene in RPAT.

The effects of OE or pair-feeding are shown in Fig. 1. The data are presented as percent values, versus controls, of the fmoles of each gene-specific mRNA found in the whole fat pad, irrespective of its actual size. Because both OE and PF treatments resulted in the shrinking of WAT masses, the relative size of the resulting fat pads is also presented as a percentage of controls' weight for comparisons.

The patterns of gene expression for OE and PF were fairly similar in EAT, and, to a lower extent in SCAT, the differences being more marked in RPAT and, especially, in MAT.

Oleoyl-estrone tended to decrease the tissue lipogenic enzyme gene expressions in MAT with respect to controls, but those in the whole tissue were somewhat higher than

Table 1 WAT sites weight and cellularity of overweight male rats treated for 10 days with oral OE or pair-fed compared with controls

Parameter (units)	Group	Mesenteric	Subcutaneous	Epididymal	Retroperitoneal
Tissue weight (g)	C	2.47 ± 0.16	2.34 ± 0.24	7.54 ± 0.65	9.07 ± 0.92
	PF	1.56 ± 0.22*	1.58 ± 0.05*	4.43 ± 0.47*	5.01 ± 0.67*
	OE	1.57 ± 0.14*	1.64 ± 0.12*	5.25 ± 0.19*	4.98 ± 0.51*
DNA content (μg/g)	C	618 ± 50^{A}	335 ± 33^{BC}	334 ± 12^{B}	255 ± 20^{C}
	PF	960 ± 85*^{A}	555 ± 35*^{B}	381 ± 4*^{C}	390 ± 49*^{D}
	OE	902 ± 50*^{A}	484 ± 42*^{B}	374 ± 22^{C}	288 ± 24^{D}
Total RNA content (μg/g)	C	137 ± 9^{A}	195 ± 8^{B}	52 ± 4^{C}	80 ± 8^{D}
	PF	319 ± 15^{A}	239 ± 5*^{B}	75 ± 4*°^{C}	138 ± 19*^{D}
	OE	274 ± 22^{A}	208 ± 15^{B}	61 ± 3^{C}	158 ± 15*^{D}
mRNA content (fg/cell)	C	41 ± 5^{AB}	93 ± 12^{C}	29 ± 3^{B}	63 ± 9^{AC}
	PF	77 ± 9*^{A}	65 ± 8^{A}	28 ± 2^{B}	58 ± 9^{A}
	OE	76 ± 6*^{A}	65 ± 4^{A}	26 ± 2^{B}	110 ± 10^{C}
Cell number in tissue (×10^{6})	C	274 ± 10	150 ± 10	410 ± 29	371 ± 17
	PF	253 ± 16	157 ± 14	300 ± 30*	291 ± 25*
	OE	263 ± 10	139 ± 14	351 ± 26	247 ± 24*
Mean cell weight (ng)	C	8.1 ± 0.6^{A}	16.0 ± 1.0^{B}	15.3 ± 0.6^{B}	20.8 ± 1.6^{C}
	PF	5.5 ± 0.5*^{A}	9.2 ± 0.5*^{B}	13.2 ± 0.1*^{C}	14.2 ± 2.1*^{C}
	OE	5.5 ± 0.4*^{A}	10.3 ± 0.7*^{B}	13.1 ± 0.7*^{C}	16.9 ± 1.1^{D}
RNA/DNA ratio (×1,000)	C	210 ± 22^{A}	610 ± 49^{B}	158 ± 12^{A}	322 ± 27^{C}
	PF	289 ± 19*^{A}	442 ± 30*^{B}	196 ± 10*°^{C}	420 ± 34*^{B}
	OE	341 ± 20*^{A}	439 ± 29*^{B}	160 ± 5^{C}	563 ± 51*^{B}

Data with different superscript letters in the same row are significantly different ($P < 0.05$; one-way ANOVA and post hoc Bonferroni test)

The values are the mean ± SEM of eight different animals

C controls, *OE* OE-treated-group; *PF* pair-fed group

Statistical significance of the differences between groups: *$P < 0.05$ versus controls; °$P < 0.05$ of OE versus PF

those expected from the reduced fat pad mass. Fatty acid transport protein 1 gene expression increased versus controls. All other transporter genes showed similar expression values to those of controls, which means that their total tissue expression was higher than that expected from a smaller fat pad size. The hormone-sensitive lipase gene showed the same pattern, but lipoprotein lipase, with lower gene expression than controls, showed little change when the mass of adipose tissue was taken into account. OE markedly decreased adiponutrin gene expression but increased that of adipose triacylglycerol lipase. The pattern elicited by pair-feeding reversed that of OE for lipogenic enzyme genes, and showed a trend towards higher expression versus controls for fatty acid transporter, lipoprotein lipase and adipose triacyl-glycerol lipase gene expression.

In contrast with the apparently enhanced metabolic activity of MAT, SCAT showed a pattern fairly in line with its reduced tissue mass, with grossly decreased lipogenic gene expressions in OE rats, and the same pattern of MAT (but at a lower setting) for fatty acid transport and binding proteins, carnitine-palmitoleoyl transferase, and lipases' gene expressions; the main difference being the lack of changes versus controls of adipose triacylglycerol lipase gene expression. PF resulted in a closely similar pattern to that described for OE, with only marked differences for acetyl-CoA carboxylase gene, which increased its expression in PF versus OE. The pattern of EAT was practically coincident with that of SCAT for OE rats; the PF animals showed no differences with respect to OE in total specific mRNA organ contents (except for higher acetyl-CoA carboxylase gene expression), despite a more marked loss of tissue weight.

The RPAT again repeated a similar pattern for OE-driven changes in gene expression, with lower than control lipogenic, enhanced fatty acid transporting and lowered lipase gene expressions, except for the adipose triacylglycerol lipase gene. The main difference with respect to the other WAT sites may be the much increased expression of the carnitine-palmitoleoyl transferase gene. In PF rats, gene expression was lower than in OE in fatty acid binders/transporter genes.

Table 3 shows the expression of the SREBP1c gene in the four WAT sites. No changes were observed with respect to controls in MAT, but in all the other WAT sites, both OE and PF resulted in significantly decreased

Table 2 Gene expression of lipid-handling proteins (fmol of the corresponding mRNAs) in the four WAT locations studied of control rats

Gene	Symbol	Units	Mesenteric	Subcutaneous	Epididymal	Retroperitoneal
ATP citrate lyase	Acly	fmol/g	0.58 ± 0.13^{AB}	0.47 ± 0.06^{AB}	0.39 ± 0.02^{A}	0.76 ± 0.16^{B}
		fmol	*1.42 ± 0.34*	*1.01 ± 0.14*	*3.47 ± 0.44*	*8.00 ± 2.63*
Acetyl-coa carboxylase α	Acaca	fmol/g	0.33 ± 0.05^{A}	0.35 ± 0.04^{A}	0.29 ± 0.02^{A}	0.43 ± 0.08^{A}
		fmol	*0.74 ± 0.09*	*0.89 ± 0.13*	*2.44 ± 0.42*	*3.79 ± 0.55*
Fatty acid synthase	Fasn	fmol/g	7.4 ± 1.3^{A}	4.3 ± 0.7^{B}	2.6 ± 0.4^{B}	7.3 ± 1.2^{A}
		fmol	*15.8 ± 2.8*	*10.6 ± 2.0*	*20.2 ± 3.5*	*63.3 ± 7.9*
Glycerol-3-phosphate acyltransferase, mit.	Gpam	fmol/g	0.40 ± 0.05^{A}	0.25 ± 0.04^{B}	0.30 ± 0.02^{AB}	0.64 ± 0.08^{C}
		fmol	*0.94 ± 0.12*	*0.54 ± 0.09*	*2.52 ± 0.32*	*6.51 ± 1.16*
Fatty acid transport protein 1	Fatp1	fmol/g	0.40 ± 0.05^{A}	0.37 ± 0.03^{A}	0.19 ± 0.03^{B}	0.35 ± 0.04^{A}
		fmol	*0.95 ± 0.13*	*0.82 ± 0.09*	*1.55 ± 0.30*	*3.57 ± 0.73*
Fatty acid translocase	Cd36	fmol/g	7.3 ± 0.9^{A}	13.0 ± 1.5^{B}	5.4 ± 0.6^{A}	11.9 ± 1.6^{B}
		fmol	*17.1 ± 2.2*	*32.8 ± 5.7*	*42.2 ± 6.6*	*105 ± 11*
Fatty acid-binding protein 4	Fabp4	fmol/g	118 ± 21^{AB}	93 ± 9^{A}	80 ± 5^{A}	180 ± 24^{B}
		fmol	*270 ± 45*	*222 ± 30*	*632 ± 73*	*1,686 ± 210*
Carnitine-palmitoleoyl transferase 1β	Cpt1b	fmol/g	0.35 ± 0.06^{AB}	0.37 ± 0.04^{A}	0.20 ± 0.01^{B}	0.40 ± 0.07^{A}
		fmol	*0.81 ± 0.14*	*0.86 ± 0.14*	*1.70 ± 0.27*	*3.43 ± 0.33*
Lipoprotein lipase	Lpl	fmol/g	16.9 ± 1.7^{A}	21.8 ± 2.7^{A}	11.3 ± 1.7^{B}	22.2 ± 3.4^{A}
		fmol	*35.9 ± 5.2*	*52.8 ± 8.0*	*84.8 ± 11.3*	*232 ± 26*
Lipase, hormone-sensitive	Lipe	fmol/g	6.1 ± 1.1^{AB}	6.2 ± 0.9^{AB}	4.6 ± 0.5^{A}	6.9 ± 0.9^{B}
		fmol	*13.4 ± 1.7*	*15.6 ± 2.5*	*36.4 ± 6.2*	*68.3 ± 10.9*
Adiponutrin	Adpn	fmol/g	1.20 ± 0.29^{A}	0.70 ± 0.11^{A}	0.56 ± 0.08^{A}	0.93 ± 0.18^{A}
		fmol	*2.73 ± 0.41*	*1.65 ± 0.25*	*4.50 ± 0.88*	*8.20 ± 1.19*
Adipose triacylglycerol lipase	ATGL	fmol/g	12.6 ± 1.5^{AB}	10.5 ± 1.3^{A}	12.6 ± 1.5^{AB}	16.7 ± 2.5^{B}
		fmol	*29.6 ± 4.1*	*23.9 ± 3.6*	*95.7 ± 13.5*	*157 ± 24*

The values are the mean ± SEM of eight different animals

Data with different superscript letters in the same row are significantly different ($P < 0.05$; one-way anova and post hoc Bonferroni test)

expression of the gene when referred to the whole fat pad mass.

Discussion

The present data show that different WAT sites actually show different metabolic patterns even for a function that is assumed constant and primary for this tissue: the management of fat reserves. The decreased cell size but unchanged number of cells (except in RPAT), suggest a limited role of apoptosis after 10 days of OE treatment, in contrast with our previous findings of active loss of cells in the short term in young female rats [39]. This was reinforced by the analysis of the ratios of Bcl2/Bax expression in a number of WAT sites, which yielded similar values in all sites; nevertheless, they were lower (not statistically significant differences) in OE-treated rats [unpublished results].

Loss of body weight in PF and OE were similar, which agrees with our previous findings that lower food intake is largely responsible for the loss of weight [38]. However, the different regulation of metabolic pathways [35] strongly suggests that OE-induced changes are largely independent of food intake [34, 38]. The powerful slimming effects of OE have been attested in rats [13] and humans [3], always inducing a marked loss of appetite and body fat, with no additional secondary or lasting effects [1].

The marked differences in mean cell size can be attributed in part to actually different adipocyte sizes [9, 16], but also to a different percentage of smaller non-lipid carrier cells (stem, endothelial, macrophages, etc.) [2], which reduce the mean cell weight when computed from tissue DNA content. In any case, the ample differences in mean size suggest that probably adipocyte size is also different [23], with larger cells showing slower metabolic responses [16, 43] and thus a higher functional inertia to change. Thus, it can be expected that EAT and RPAT will show lower protein expression per unit of weight than small-cell WATs such as MAT and SCAT; since in these sites mRNA content per gram of tissue was, respectively, 4.6 and 6.0 versus 1.6 and 2.6 μg/g of EAT and RPAT. These differences contrast with the fairly uniform values of

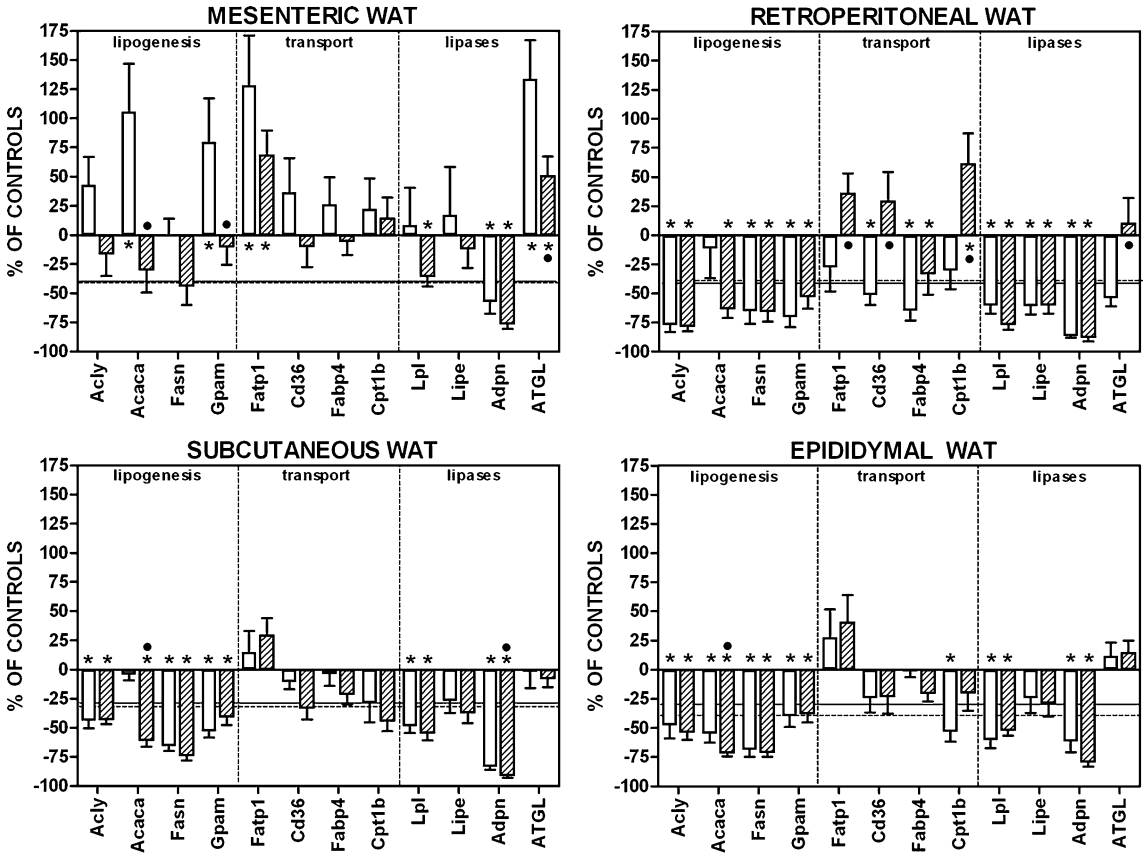

Fig. 1 Patterns of gene expression of lipid-handling proteins in four WAT sites of OE- and pair-fed-treated rats. The values are the mean ± SEM of eight different animals, and are expressed as percentages, versus the control values of the total amount (fmol) of the given transcript in the whole fat pad. *White columns* pair-fed; *dashed columns*: OE. The *horizontal line* at 0 represents the 100% value of controls' transcript content. The *fine horizontal dashed line* corresponds to the mean percentage of the specific site weight (g) of PF rats versus the corresponding controls; the *continuous line* corresponds to the percentage of OE vs. controls' weight. An *asterisk* indicates a significant ($P < 0.05$) difference of the data with respect to controls, and a *black dot* represents a significant ($P < 0.05$) difference between matching OE and PF groups (one-way anova and post hoc Bonferroni test)

Table 3 Gene expression (fmol of the corresponding mRNAs) of SREBP1c in the four WAT locations studied of control rats

Gene	Symbol	Group	Mesenteric	Subcutaneous	Epididymal	Retroperitoneal
Sterol regulatory element binding factor 1	Srebf1	C	2.22 ± 0.33	3.55 ± 0.68	6.83 ± 1.22	9.86 ± 1.76
		PF	3.23 ± 0.68	1.92 ± 0.27*	3.11 ± 0.54*	2.38 ± 0.76*
		OE	3.00 ± 0.38	1.86 ± 0.28*	3.44 ± 0.46*	4.57 ± 0.79*

The values are the mean ± SEM of eight different animals, and are expressed as fmol of SREBP1c mRNA per gram of tissue

Statistical significance of the differences between groups: *$P < 0.05$ versus controls (Student's t test)

There were no statistical differences between PF and OE groups

C controls, *OE* OE-treated-group, *PF* pair-fed group

the gene expressions per unit of tissue weight in the four sites, which hints at a special preservation of the lipid metabolism functions studied under severe energy drainage: the 'share' of mRNA resulting from these genes' expression constituted a larger share of total mRNA than in cells under normal conditions and with higher total mRNA.

The fat mobilizing effect of OE has been found not to depend solely on decreased food intake [32]. By design, pair-feeding equals the decreases in food intake of the OE-treated animals, but OE also induces a severe drain in body reserves [29] imposed by the maintenance of energy expenditure [40, 41]. Despite similar effect on WAT lipid mobilization found here, OE effects on circulating glucose, fatty acids and other energy parameters are much milder than those elicited by food deprivation, including pair-feeding [38], which attests to a different handling of the

reserves by other tissues and a lower degree of metabolic stress by OE under identical energy availability decreases [38].

Oleoyl-estrone induced a marked decrease in lipogenesis in all WAT sites with the exception of MAT; PF resulted in a similar pattern, but a key lipogenic enzyme gene expression (acetyl-CoA carboxylase) did not change, in agreement with previous reports [20]; thus, consistently showing higher values than in OE, hinting at different mechanisms (or intensity) of inhibition of lipogenic gene expression in either group.

SREBP1c is a powerful lipogenic enhancer [20, 43], activated by energy availability, insulin [11], and a number of lipogenic signals [42]. We have found recently that SREBP1c upregulates liver lipogenesis under OE treatment [33], a probable consequence of the maintenance of glycemia and glucose availability in OE rats [8, 13] in contrast with semistarved or pair-fed animals [38]. The fall in SREBP1c expression, observed here for SCAT, EAT and RPAT, fully agrees with their markedly decreased lipogenic enzyme gene expressions. In MAT, the lack of overall OE-induced changes in lipogenic gene expressions was paralleled by a similar lack of changes in the expression of the SREBP1c gene, but the actual increase of some lipogenic enzyme gene expressions induced by PF was not correlated with changes in the expression of SREBP1c. This difference suggests that factors additional to SREBP1c may influence the observed effects of PF on lipogenic gene expression; however, the more consistent effects of OE were closely correlated with this regulator, which further supports its probable implication on the mechanism of action of OE [33].

Lipoprotein lipase gene expression was diminished in OE rats, in agreement with the OE-elicited decrease in its activity observed in Zucker obese rats [4], and is justified by the direction of fatty acid flow from WAT to other tissues, as in starvation [24]. The marked decrease in the expression of adiponutrin in OE and PF was again correlated with the assumed decrease in lipogenesis, because of the association of this lipase with the lipogenic process [19].

Hormone-sensitive lipase plays a key role in the mobilization of the WAT fat stores [37]. The expression of the hormone-sensitive lipase gene was lower than in controls in all sites in OE and PF rats, but the values were similar to those of controls when expressed per gram of tissue. Thus, this gene was not affected by OE, which agrees with its regulation via kinases and not through transcription [14]; this stability helps us to assess by contrast the deep changes experienced in the expression of other genes. Its lack of expression changes with respect to tissue weight, together with the relative increases observed in all sites in the expression of the adipose triacylglycerol lipase gene, may help explain that, overall, lipolytic gene expressions relatively surpassed the lipogenic ones (when compared with controls) in OE and PF, which is in agreement with the wasting of WAT, the predominance of lipolysis and the constant flow of fatty acids from WAT in rats under OE treatment. This is compounded by the eventual intervention of the adrenergic-driven cAMP cascade regulation of hormone-sensitive lipase (expression unchanged), since OE increases cAMP availability [7]. Increased expression of the carnitine-palmitoleoyl transferase gene in the RPAT of OE rats also suggests that at least part of the lipid lost may be used for energy maintenance in the tissue itself.

A large part of the process of reduction of fat stores elicited by OE is comparable to that induced by pair-feeding, suggesting that the main factor driving the loss of WAT fat is the response to low energy availability, probably mediated by changes in catecholamine and leptin signaling. This similarity of mechanisms hints at a common regulation of lipolysis, that is not coincident with that of lipogenesis, and this difference may mark the dissimilitude between the exhaustion of WAT energy under normoglycemia characteristic of OE [13] with the mobilization parallel to hypoglycemia typical of limited feeding [38].

The MAT was, again, peculiar in its pattern of expression changes induced by OE and PF. Despite an overall shrinking of body WAT (and that of its triacylglycerol stores) [11, 28], with highly increased lipase expressions and even higher fatty acid handling protein genes', lipogenesis was not inhibited by OE, in contrast with the other WAT sites. The picture was the same for PF (but with higher gene expression values throughout), suggesting a common origin and regulation. This difference may stem from the privileged position of MAT, the only truly anatomically visceral WAT, receiving substrates from the intestine and voiding its metabolic proceeds through the portal vein directly to the liver. Probably the assumed high-lipogenic activity and active handling and transport of lipids may be related to the synthesis of nascent lipoproteins and the integration of fatty acids derived from intestinal absorption into these lipoproteins [18]. The relatively low-lipid content of MAT [28], small mean cell size (i.e. suggesting the presence of a large number of non-adipocyte cells, and smaller, and thus more active, adipocytes), and a marked enhancement in the expression of intracellular fatty acid-binding and compartment shifting protein genes, suggests a rapid turnover of lipids that contrasts with the more or less long-term storage and paucity of metabolism of the other WAT sites studied.

In conclusion, the data presented suggest that OE effects on WAT may be more a consequence of inhibited lipogenesis than enhanced lipolysis, an effect fully comparable to that produced by pair-feeding. There is a considerable coincidence on the patterns of expression of lipid-handling

protein genes of WAT in different sites for OE or PF rats, supporting largely shared mechanisms of fat mobilization. The four sites tested show different lipid-handling gene expression patterns, hinting at different functions / regulation sensitivity that apply also to the tissue-defining fat storage role, in addition to their known diversity in other signaling and hormonal functions [2, 17, 36]. Thus, the generalization of conclusions from the analysis of WAT samples from a single site cannot be extended to the whole body WAT because of the differences are present even in such a common and basic function of WAT as is lipid energy storage. Our results are in line with the previous studies on WAT site-related differences in gene expression and the response to pharmacological or physiological challenges [28, 30, 31, 40].

A fair conclusion derived from this study is the marked metabolic differences between MAT and the other WAT sites studied. Truly, MAT is the best representative example of 'visceral' fat depot, different from all others because of its direct relationship with the intestinal circulation and portal venous drainage. However, the differences between SCAT and RPAT or EAT were considerably smaller, and could not sustain the common practice of considering 'visceral' all intra-abdominal depots (such as RPAT or EAT) as counterposed to peripheral depots such as SCAT. The data presented here should be taken as a further contribution to the strict differentiation of WAT function depending on anatomy, blood supply, and drainage and not only on general visceral or subcutaneous location.

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