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Oleoyl-estrone affects lipid metabolism in adrenalectomized rats treated with corticosterone through modulation of SREBP1c expression

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ABSTRACT

Oleoyl-estrone (OE) elicits a decrease in body fat, which is blocked by glucocorticoids. In order to analyze this counterregulatory effect, we studied the effects of oral OE on adrenalectomized female rats simultaneously receiving corticosterone (subcutaneous pellets). Circulating corticosteroids, liver glycogen, lipids and the expressions in whole liver, soleus muscle, interscapular brown adipose tissue (BAT), and the inguinal and periovaric white adipose tissue (WAT) of genes controlling lipid metabolism were analyzed. Corticosterone reversed OE lipid mobilization, storing fat in liver and subcutaneous WAT. This was not simply the predominance of corticosteroid enhancement of lipogenesis against OE inhibition, but a synergy to enhance lipogenesis. Periovaric WAT showed a different effect, with corticosterone inhibiting OE arrest of lipogenic gene expressions. The data presented suggests that interaction of OE and glucocorticoids (and the metabolic response) depends on the organ or WAT site; there was a direct relationship on the direction and extent of change of SREBP1c expression with those of important energy and lipid handling genes. Our results confirm that corticosterone blocks – and even reverses – OE effects on body lipids in a dose-dependent way, a process mediated, at least in part, by modulation of SREBP1c expression.
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1. Introduction

Oleoyl-estrone (OE) treatment rapidly decreases body fat stores sparing protein [1]. OE mobilization of lipid specially affects white adipose tissue (WAT), by decreasing its mass [2] through apoptosis [3] and massive loss of lipid [1,2], accompanied by a decrease in food intake and the maintenance of energy expenditure [4]. OE effects have been observed in a number of animals models, including genetic [5,6] or dietary [7,8] models of obesity, even using hyperlipidic diets [9]. In addition to the modification of energy balance, OE also decreases insulin resistance and markedly lowers cholesterol levels, improving hyperlipidemia [10].

OE elicits the secretion of ACTH and corticosterone in rats [11], a process not directly related to changes in hypothalamic CRH [12]. OE effects are more intense in animals lacking functional adrenal glands [13], an effect that is reversed by corticosterone administration [14].

Glucocorticoid action has been directly related to the development and maintenance of the metabolic syndrome [15], since they can induce insulin resistance [16], increase blood lipids [17] and elicit the hepatic insulin resistance characterized by the enhancement of hepatic glucose output [18]. In addition, the stimulation of the HPA axis increases WAT proliferation [19], and pro-inflammatory cytokine production [20], which are in the basis of the pathogenic derangement of the carbohydrate-lipid energy homeostasis maintained by insulin that characterizes diabetes [21].

The frank inhibition by glucocorticoids of the slimming ability of OE is partly mediated by the effects of both on the glucocorticoid activity-enhancing enzyme 11β-hydroxysteroid dehydrogenase (type 1) [22] and on free circulating glucocorticoid levels, through modulation of CBG expression [23], in a way that OE enhances the glucocorticoid activity that in turn tends to block or reverse OE activity [14]. This reversion is fairly logic, since glucocorticoids and OE exert opposite actions on energy substrate handling, and on the metabolic control of glycemia and lipidemia. In a previous paper we have found that glucocorticoids induce an "OE-resistance" comparable to the glucocorticoid-induced insulin resistance [14]; glucocorticoids affect the expression and regulation of the insulin

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signal transduction cascade [24] inducing as well indirect actions such as modulation of glucose and fatty acid availability resulting in altered responses to glucose [25,26]. In the case of OE, which mechanism of action is just beginning to be unveiled, no such comparisons are possible. However, the analysis of a number of energy metabolism genes in organs related to energy wasting (muscle, brown adipose tissue), storage (WAT) and distribution (liver) may give us some insight about how OE elicits its energy control function through regulation of the expression of key enzymes and regulating agents. OE treatment on liver results in a partial increase of lipogenesis, a process at least in part mediated by SREBP1c [27], a well known signal transduction modifying agent [28,29] which modulates the effects of a number of steroid and peptide hormones [30,31] on their action upon the lipogenic pathway.

Since corticosterone strongly inhibited (and even reversing) the overall lipid wasting effects of OE [14], in a dose-dependent way, we intended here to determine whether the mechanism of this interference was related to the modulation of the lipogenic pathway via SREBP1c, as a way to better understand the mode of action of OE and its interaction with glucocorticoids.

2. Materials and methods

2.1. Animals and experimental setup

Female Wistar rats (Harlan-Interfauna, Sant Feliu de Codines, Spain) weighing 210–230 g were used. After acclimation to the animal house, the animals were adrenalectomized or sham-operated under isoflurane anesthesia (day −5) and left to recover for 5 days. Bilateral removal of the adrenals was achieved through two small dorsolateral skin incisions; the glands were pulled out by holding the periadrenal fat and then were excised. Sham-operated animals were handled in the same way as the adrenalectomized except that the adrenal glands were not cut and removed.

All animals had free access to pellet food (maintenance chow, Panlab, Barcelona Spain) and tap water; adrenalectomized rats had the water substituted by a saline solution (9 g/L NaCl). All rats were kept in individual cages in a light (12 h on, from 08:00/12 h off), temperature (21–22 °C) and humidity (74–77%) controlled quiet environment.

The rats received a daily gavage (from days 0 to 8) of 0.2 mL sunflower oil, alone or containing OE (OED, Barcelona Spain) at a dose of 10 nmol/g of body weight per day. Corticosterone dosing was achieved by implanting subcutaneously in the back, the same day surgery (sham-operated or adrenalectomized groups, day −5) was performed, with slow-releasing cholesterol-free corticosterone pellets (Innovative Research of America, Sarasota, FL USA) which liberated the hormone continuously for 21 days. Pellets of 0 (placebo), 10, 35 and 100 mg corticosterone were used, yielding daily doses of 0, 0.48, 1.67 and 4.76 mg/day.

Half the animals (six) in each group were given an oil gavage (i.e. controls), and half the OE-containing oil gavage (i.e. OE). The groups included in the present study were (a) sham-operated rats implanted with placebo pellets (both controls and OE); (b) adrenalectomized rats implanted with placebo pellets (both controls and OE); (c) adrenalectomized rats implanted with 10 mg pellets of corticosterone (both controls and OE); (d) adrenalectomized rats implanted with 35 mg pellets of corticosterone (both controls and OE); and (e) adrenalectomized rats implanted with 100 mg pellets of corticosterone (both controls and OE). In all cases, daily body weight and food consumption were recorded.

The experimental setup and procedures were approved by the Ethics Committee of the University of Barcelona. All animal handling procedures were carried out following the guidelines established by the EU, and the Spanish and Catalan Governments.

2.2. Sample preparation and analytical procedures

On day 8, the rats were quietly taken out of their cages and killed by decapitation in less than 30 s. Blood was recovered to obtain serum, kept at −80 °C. Samples (and the weights) of liver, soleus muscle, interscapular brown adipose tissue, periovaric WAT pad and subcutaneous inguinal WAT strips were obtained, frozen with liquid nitrogen and stored at −80 °C.

Liver glycogen was estimated using a chemical method [32] after alkaline digestion and ethanol precipitation [33]. Liver lipid was extracted through homogenization of liver samples in 10 volumes of trichloromethane/methanol [34] using a Polytron (Kinematica, Luzern, Switzerland) tissue disruptor. Total lipids were estimated as previously described [34].

Serum corticosterone, dehydrocorticosterone and deoxycorticosterone were measured by HPLC–MS/MS as previously described [14] using a Varian system (Varian, Palo Alto, CA, USA).

2.3. Semiquantitative analysis of gene expression

We used a previously described semiquantitative method [35] that allows the comparison of different organs and genes through the estimation of the absolute content of specific mRNAs per unit of tissue weight or, as is the case here, in a whole organ (liver, soleus muscle) or a defined tissue site (interscapular BAT, periovaric WAT or inguinal subcutaneous WAT strips).

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). 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. The primers used for the estimation of gene expression are presented in Supplemental Table 1.

2.4. Statistical analyses

Statistical comparisons between groups were established by one- or two-way ANOVAs, and the post hoc Bonferroni test, using the Prism 5 (GraphPad, San Diego, CA, USA) program.

3. Results

3.1. Hormone levels

Table 1 shows the levels of glucocorticoids of adrenalectomized rats treated with OE and increasing doses of corticosterone. The glucocorticoid values found in sham animals were high because of the stress associated to handling and killing. Adrenalectomized rats showed undetectable corticosterone or dehydrocorticosterone; their levels increased with the corticosterone dose, with no significant effect of OE. As expected for adrenalectomized rats, deoxycorticosterone levels were low, at or below the detection limit for the method used.

Table 1
Plasma levels of adrenal steroid hormones in sham-operated and adrenalectomized rats subjected to combined treatment with OE and corticosterone.

Parameter	Group	Sham-operated	adx-0	adx-10	adx-35	adx-100	ANOVA analysis			
							adx	C dose	C dose + OE	OE
Dehydrocorticosterone (nM)	C OE	55.1 ± 12.1 48.6 ± 24.1	ND ND	15.2 ± 8 9.3 ± 3.1	29.7 ± 9.5 24.4 ± 4.9	48.1 ± 8.5 54.5 ± 11.0	0.0026	0.0051	0.0003	NS
Corticosterone (nM)	C OE	417.9 ± 165.4 461.6 ± 408.6	ND 21.5 ± 6.2	86.7 ± 18.4 138.8 ± 15.6	226.9 ± 48.6 302.9 ± 66.3	557.6 ± 58.7 551.1 ± 49.6	0.0380	0.0000	0.0000	NS
Deoxycorticosterone (nM)	C OE	0.6 ± 0.2 0.5 ± 0.4	ND ND	0.7 ± 0.0 0.2 ± 0.0	ND 0.1 ± 0.0	0.3 ± 0.0 ND	0.0256	NS	NS	NS

The values are the mean ± S.E.M. of six different animals; ND = not detected with the methodology used. Statistical significance of the differences between groups (ANOVA): adx = effect of adrenalectomy; C dose = effect of corticosterone dose on adrenalectomized rats; C dose + OE = effect of corticosterone dose on adrenalectomized rats receiving OE; OE = effect of OE on all treated rats. Differences between groups due to OE treatment (post hoc Bonferroni test): NS = not significant ($P > 0.05$).

3.2. Tissue weights

As expected, adrenalectomy did not alter the rat's body weight significantly. OE treatment alone reduced body weight, but concomitant administration of corticosterone induced a biphasic effect first increasing and then decreasing body weight. Adrenalectomy alone decreased the weight of the liver and its glycogen and lipid content (Table 2). Corticosterone treatment increased the periovaric WAT pads, the effect on the other organs studied being not significant. OE elicited a sustained reduction of periovaric and subcutaneous WAT, interscapular BAT and soleus muscle weight; however, combined OE and corticosterone increased liver weight, in part because of increased glycogen depots.

3.3. Liver gene expressions

Fig. 1 shows the effects, on SREBP1c expression, of corticosterone administration in the presence/absence of OE treatment; in all the tissues studied, data were compared with untreated sham-operated controls (i.e. receiving placebo pellets and vehicle-only gavage). Corticosterone had a limited effect on liver SREBP1c gene expression (Fig. 1A), but the presence of OE was translated in a corticosterone dose-dependent increase of its expression. The patterns of change of the relative gene expression for acetyl-CoA carboxylase 1, fatty acid synthase and citrate: ATP lyase closely followed the pattern described for SREBP1c expression. The numerical gene expression data can be found in the Supplemental Table 2.

3.4. White adipose tissue gene expressions

Subcutaneous WAT SREBP1c gene expression pattern was similar to that of liver, but the increase of its expression under OE was relatively higher. Again, lipogenic enzyme gene expressions closely followed the SREBP1c pattern (Fig. 1B, and Supplemental Figure 1). The numerical gene expression data can be found in Supplemental Table 3. In contrast with subcutaneous WAT, in periovaric WAT corticosterone increased SREBP1c expression; OE had a marked inhibitory effect on adrenalectomized rats, which was promptly overcome by corticosterone, except at the highest dose (Fig. 1B). Lipid synthesis enzyme also showed patterns of gene expression similar to those for SREBP1c (Fig. 1B, and Supplemental Figure 2). The numerical gene expression data can be found in Supplemental Table 4.

3.5. Brown adipose tissue gene expressions

In interscapular BAT, corticosterone hardly altered the expression of SREBP1c gene, pattern closely mimicked by lipogenic

Table 2
Body and tissues weight and composition changes in sham-operated and adrenalectomized rats subjected to combined treatment with OE and corticosterone.

Parameter	Group	Sham-operated	adx-0	adx-10	adx-35	adx-100	ANOVA analysis			
							adx	C dose	C dose + OE	OE
Rat weight (g)	C OE	236 ± 11 231 ± 11	228 ± 3 211 ± 7	231 ± 3 218 ± 5	230 ± 3 225 ± 8	222 ± 3 208 ± 7	NS	NS	0.0025	0.0105
Liver weight (g)	C OE	7.28 ± 0.28 8.12 ± 0.48	6.09 ± 0.37 5.19 ± 0.50	6.70 ± 0.24 7.71 ± 0.31	6.69 ± 0.26 7.48 ± 0.52	6.61 ± 0.37 8.47 ± 0.50	0.0003	NS	0.0209	0.0074
Soleus muscle weight (each) (mg)	C OE	114 ± 7 104 ± 5	126 ± 17 97 ± 1[o]	113 ± 2 106 ± 6	109 ± 6 105 ± 5	106 ± 3 98 ± 6	NS	NS	0.0326	0.0185
Interscapular BAT mass (mg)	C OE	372 ± 29 251 ± 15	326 ± 33 200 ± 9[o]	323 ± 17 268 ± 31	314 ± 36 223 ± 44	312 ± 33 306 ± 36	NS	NS	0.0042	0.0002
Periovaric WAT mass (both sides) (g)	C OE	3.22 ± 1.15 2.58 ± 0.94	1.95 ± 0.48 1.85 ± 0.37	2.51 ± 0.53 2.69 ± 0.74	4.38 ± 1.17 2.59 ± 0.42	4.58 ± 0.27 2.19 ± 0.39	NS	0.0279	0.0140	0.0395
Inguinal subcutaneous WAT mass (both sides) (g)	C OE	1.55 ± 0.41 0.90 ± 0.36	0.91 ± 0.14 0.82 ± 0.15	1.27 ± 0.17 1.04 ± 0.07	1.45 ± 0.28 1.15 ± 0.08	1.62 ± 0.16 1.31 ± 0.11	NS	NS	0.0293	0.0207
Liver lipids (mg)	C OE	240 ± 9 257 ± 15	159 ± 10 161 ± 15	225 ± 8 254 ± 10	240 ± 9 240 ± 17	273 ± 15 299 ± 18	0.0000	0.0000	NS	NS
Liver glycogen (mg)	C OE	313 ± 12 283 ± 17	205 ± 12 146 ± 14[o]	242 ± 9 310 ± 13[o]	238 ± 9 282 ± 19	189 ± 11 282 ± 17[o]	0.0000	0.0000	0.0007	0.0120

The values are the mean ± S.E.M. of six different animals. Statistical significance of the differences between groups (ANOVA): adx = effect of adrenalectomy; sham + OE = effect of OE on sham-operated rats; adx-OE = effect of OE on adrenalectomized rats; C dose = effect of corticosterone dose on adrenalectomized rats; C dose + OE = effect of corticosterone dose on adrenalectomized rats receiving OE; OE = effect of OE on all treated rats. NS = not significant ($P > 0.05$).

[o] Differences between groups due to OE treatment (post hoc Bonferroni test): $P < 0.05$.

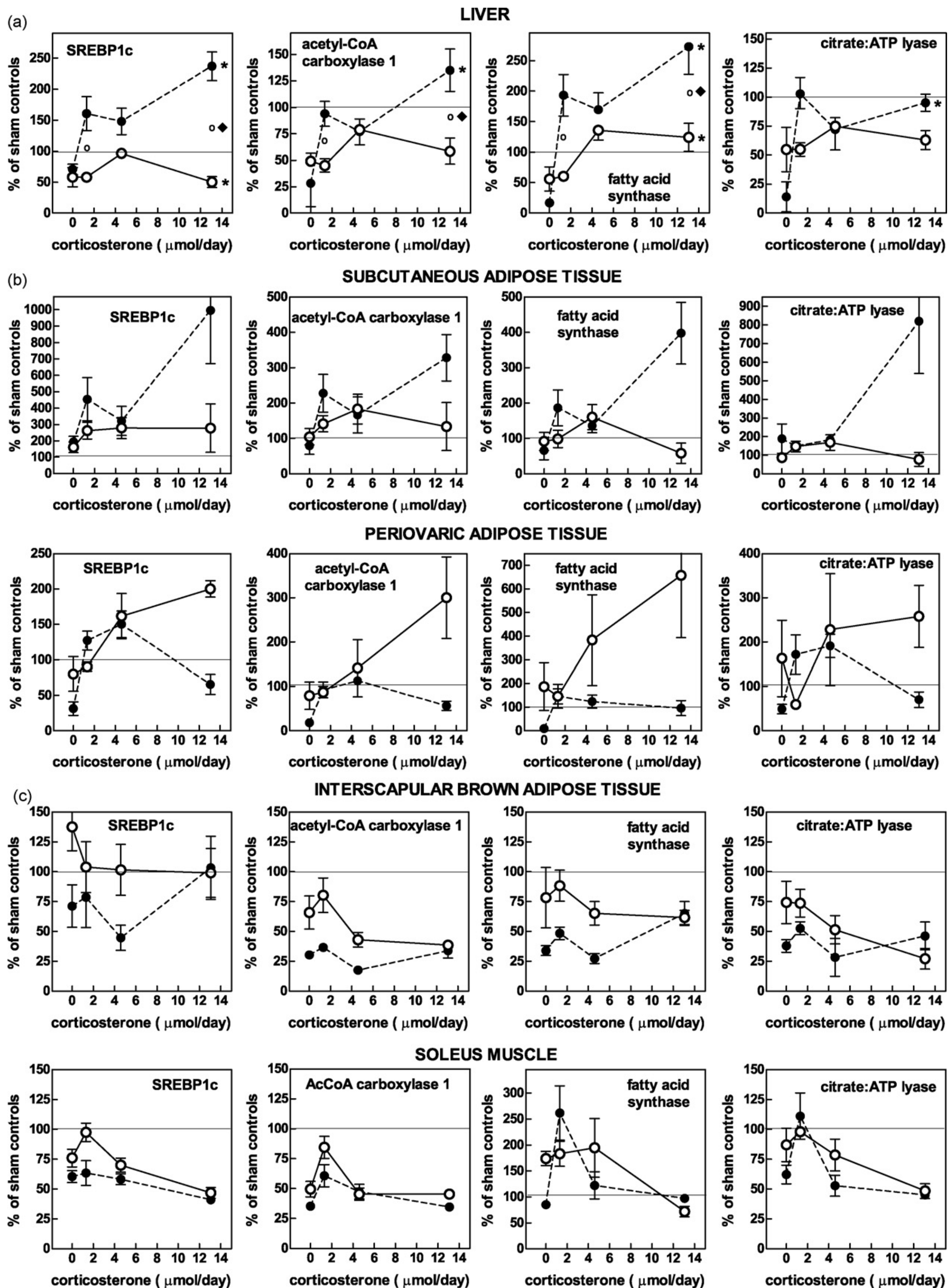

Fig. 1. SREBP1c, acetyl-CoA carboxylase 1, fatty acid synthase and citrate: ATP lyase gene expressions in liver (A), subcutaneous and periovaric white adipose tissues (B), interscapular brown adipose tissue and muscle (C) of adrenalectomized female rats treated with different doses of corticosterone and oral OE. All data are presented as percentage of the mean values for sham-operated controls' content of the corresponding gene mRNA in the whole organ (horizontal 100% line). Controls: white dots and continuous line; OE: black dots and dashed line. Statistical significance of the differences between groups: *$P < 0.05$ versus (sham) controls; °$P < 0.05$ between corticosterone and corticosterone + OE groups. A diamond indicates an overall (two-way ANOVA) statistical significance of the differences between both treatments.

Table 3
Statistical significance of the correlations between the pattern of SREBP1c expression and those of lipid/energy-related enzyme gene expression patterns in key organs of adrenalectomized rats treated with corticosterone and OE.

Lipid/energy enzyme gene expression	Liver		Soleus muscle		Interscapular BAT		Periovaric WAT		Subcutaneous WAT	
	C	C + OE	C	C + OE	C	C + OE	C	C + OE	C	C + OE
Acetyl-CoA-carboxylase 1										
r^2	0.5390	0.6956	0.5841	0.3106	0.3236	0.4963	0.3149	0.5118	0.8204	0.6858
P	0.0018	0.0000	0.0001	0.0132	0.0137	0.0008	0.0368	0.0018	0.0000	0.0000
Acetyl-CoA-carboxylase 2										
r^2	0.7122	0.5651	0.5233	0.4830	0.0056	0.0067	–	–	–	–
P	0.0000	0.0003	0.0005	0.0007	NS	NS				
Citrate: ATP lyase										
r^2	0.6785	0.7986	0.4054	0.2507	0.0634	0.1236	0.1819	0.8187	0.4997	0.9080
P	0.0000	0.0000	0.0034	0.0245	NS	NS	NS	0.0000	0.0009	0.0000
Fatty acid synthase										
r^2	0.5753	0.6871	0.3517	0.2684	0.3887	0.5186	0.2545	0.2181	0.5119	0.6979
P	0.0007	0.0000	0.0074	0.0231	0.0057	0.0005	NS	NS	0.0008	0.0000
Lipoprotein lipase										
r^2	–	–	0.2209	0.3112	0.2880	0.1897	0.2209	0.6408	0.1945	0.2523
P			0.0423	0.0106	0.0217	NS	0.0423	0.0001	NS	0.0336
Hormone-sensitive lipase										
r^2	–	–	–	–	–	–	*0.3513*	*0.4899*	*0.2321*	*0.0893*
P							0.0255	0.0018	0.0429	NS
Adiponutrin										
r^2	–	–	–	–	–	–	0.1246	0.3969	0.8321	0.2472
P							NS	0.0067	0.0000	0.0423
Carnitine-palmitoleoyl transferase										
r^2	–	–	0.4231	0.1762	0.0980	0.0059	–	–	–	–
P			0.0026	NS	NS	NS				
Acyl-CoA dehydrogenase (medium chain)										
r^2	–	–	0.0604	0.0948	0.5800	0.6207	–	–	–	–
P			NS	NS	0.0002	0.0000				
Glucose transporter 4										
r^2	–	–	0.4403	0.4409	0.3054	0.6214	0.4602	0.3699	0.7941	0.2692
P			0.0037	0.0014	0.0214	0.0000	0.0054	0.0096	0.0000	0.0273
Uncoupling protein 1										
r^2	–	–	–	–	0.3631	0.4064	–	–	–	–
P					0.0105	0.0033				

The data represent the statistical significance of the correlation coefficients of the given gene expression patterns compared with the matching groups of SREBP1c expression (as shown in Supplementary Tables 2–6) in the same organ/tissue (ANOVA); NS = not significant ($P > 0.05$); (–) not measured. C = corticosterone alone; C + OE = simultaneous corticosterone and OE treatment.

enzyme gene expressions (Fig. 1C, and Supplemental Figure 3). The addition of OE to corticosterone resulted in a marked inhibition of SREBP1c expression, at least at low corticosterone doses. The numerical gene expression data can be found in Supplemental Table 5.

3.6. Soleus muscle gene expressions

Finally, in soleus muscle, a biphasic effect of corticosterone was observed: the lowest dose recovered the normal expression of SREBP1c from decreased adrenalectomized rats' values, but increasing corticosterone doses further decreased the gene expression (Fig. 1C). OE treatment followed a closely similar pattern, but expression levels were maintained throughout at only a fraction of the expression of sham-operated controls. This pattern of change was closely followed by the expression of lipogenic enzymes (Fig. 1C and Supplemental Figure 4). The numerical gene expression data can be found in Supplemental Table 6.

3.7. Gene expression correlations

In sum, the patterns described for SREBP1c were almost identical to most of the lipogenic and other energy handling enzyme genes studied, with a distinct pattern of change elicited by corticosterone plus or minus OE essentially depending on the organ studied. The close similarity of the patterns observed and their likeness to those described in Fig. 1 for SREBP1c expression are summarized in Table 3, in which we present the correlation coefficients found between the individual values for each rat between SREBP1c expression in the whole tissue/organ presented as a percentage of sham-operated controls' values and those representing the expression of each gene for lipid handling enzymes.

In liver, the degree of correlation between the expression of lipogenic enzyme genes and that of SREBP1c was highly significant in all cases. In soleus muscle, this correlation extended to GLUT4 and lipoprotein lipase, but there was no correlation with the expression of acyl-CoA dehydrogenase, which showed no changes; carnitine-palmitoleoyl-transferase gene expression showed a significant correlation only in the absence of OE. In interscapular BAT, corticosterone did not affect lipogenesis, following the SREBP1c expression pattern; the mitochondrial disposal of fatty acids was also potentially decreased. In the case of OE-treated animals citrate: ATP lyase (neither correlated in controls), lipoprotein lipase, and carnitine palmitoleoyl-transferase patterns were not significantly comparable to those of SREBP1c.

In the adipose tissues, the similitudes of expression patterns of lipogenic enzymes with those of SREBP1c were also high, the main difference being found in periovaric (but not subcutaneous) WAT,

where the patterns observed for fatty acid synthase and citrate: ATP lyase gene expression showed differences with SREBP1c expression pattern.

4. Discussion

Using the same experimental setup described here we have previously observed that the combination of OE and corticosterone induces a marked loss of OE-dependent energy wasting [14]. OE maintained glycemia and low insulin levels, with a marked decrease in circulating cholesterol. OE alone also induces a marked reduction of adipose tissue lipid reserves [8] in rats, mainly through maintained lipolytic and inhibited lipogenic imbalance [27]. In adrenalectomized rats supplemented with corticosterone and treated with OE, the effects of OE alone were similar, but the added presence of corticosterone resulted in increases of circulating triacylglycerols, but not NEFA, a relative increase in cholesterol, and a small but significant rise in glucose [14]. This was paralleled by increases in insulin (over the glucocorticoid-alone values), leptin and adiponectin (both below the corticosterone alone group) [14].

OE is a powerful lipid-mobilizing agent, however, as we have recently found, glucocorticoids strongly detain and even revert this action [14]. In liver, OE increases lipogenic enzyme gene expression, but also inhibits lipogenesis in most peripheral tissues [27]. Combined treatment with OE and corticosterone results in the maintenance of liver lipogenesis but also enhancing subcutaneous WAT lipogenic gene expression, which are thus dependent on corticosterone. OE effects wasting WAT energy stores were markedly different in the periovaric and subcutaneous sites. The effect observed is not a simple predominance of corticosteroids enhancing lipogenesis [36] against OE inhibiting [31], which may be true for subcutaneous WAT, in which OE effects were overcome by corticosterone (maximal increase in WAT site weight in the range of 60–80% irrespective of OE). However, in periovaric WAT, OE was able to strongly inhibit the powerful lipogenic drive of corticosterone (an increase in WAT weight of a mere 18% versus 135% of corticosterone alone). This differential behavior of two of the main WAT sites suggests a widely different role and sensitivity to hormonal change, in a way that makes the subcutaneous masses more akin to liver-like regulation, and periovaric WAT, with more abundant glucocorticoid receptors [37], behaving more as a typical storage site.

SREBP1c is a powerful lipogenic intermediary which is activated by liver X receptors [26], insulin [30], or simply by substrate availability [38]. High corticosterone doses inhibited the expression of SREBP1c in the liver. The effects of OE in liver and subcutaneous WAT SREBP1c gene expression were largely dependent on the presence of corticosterone in a synergistic way, whereas in the other tissues studied: muscle, BAT and periovaric WAT, this enhancement was not observed or even their effects were opposed. This is a complex pattern that cannot be explained by a simple direct interaction of either hormone on the synthesis of SREBP1c, but probably through a chain of events with three distinct patterns: (a) liver and subcutaneous WAT, (b) muscle and BAT and (c) periovaric WAT. This agrees with the different handling roles of liver and the WATs, depending on their location and physiological role, but the closeness of BAT and muscle is suggestive of an even closer physiological and epigenetic relationship [39].

The role of SREBP1c, as a key lipogenic activator [40], has been observed to be critical in the control of lipid availability by OE [41]. In fact, the main effect of OE on lipid metabolism, as studied on male rats, lies in the inhibition of lipogenesis in WAT – but not in liver [27] – rather than increased lipolysis or fatty acid disposal [41], and these effects are largely mediated by a strong inhibition of SREBP1c expression [42]. OE-induced changes in hepatic expression of SREBP1c have been observed to translate in directly proportional levels of the protein [27]. The presence of corticosterone deeply affected the effect of OE on SREBP1c expression in subcutaneous WAT, following the same pattern as liver and resulting in a marked increase in its expression that may be translated in enhanced lipogenesis. On the other hand, in periovaric WAT, the decrease in SREBP1c expression was countered by corticosterone, except at the highest dose, when its expression was strongly inhibited. As a consequence, the patterns of response of both WAT masses were essentially different.

In overweight male rats, most WAT sites show a similar gene expression pattern for lipogenesis enzyme genes [41], in contrast with the widely different pattern observed here for lean female rats between WAT sites; this may be a consequence of the sex-dependent effects of OE on the wasting of body energy [43].

In soleus muscle and interscapular BAT, SREBP1c expression was affected in a more uniform way: in both cases OE inhibited its expression and corticosterone also inhibited its expression in muscle but not in BAT; there were no additive or countering effects of both hormonal agents. This similar behavior, widely different from those of WAT may be related to their shared energy metabolism control systems [44], reflecting a similar cellular origin [39].

Brown adipose tissue is a peculiar adipose tissue that shares with WAT the drive to store fat, but also may waste it when needed by sympathetic control [45], which makes it closer to muscle; interscapular BAT showed a marked responsiveness to OE in the absence of corticosterone. In spite of overall thermogenesis being maintained in animals treated with OE [46], the generalized decrease in expression (and size), including UCP1 expression suggests a lowered role of BAT under OE treatment, that may be enhanced by the presence of glucocorticoids, thus reversing the lipogenic drive observed in WAT sites. The shutting off of this tissue can be followed by the marked decrease in insulin signaling, compounded by OE, with increasing corticosterone doses [14].

The analysis of a number of key enzymes' expressions responsible of the control of lipogenesis (and lipolysis) showed a markedly identical series of patterns of change under combined OE and corticosterone treatment. In liver, lipogenic enzyme expression patterns closely followed those of SREBP1c, and these similitudes were extended to the other organs studied, in spite of specific differences in organs. Key enzymes of fatty acid disposal, such as carnitine palmitoleoyl transferase or a β-oxidation enzyme: acyl-CoA dehydrogenase, followed the SREBP1c pattern only partially, as did lipoprotein lipase, or acetyl-CoA carboxylase 2, essentially regulative [47], but not the 1 form, of lipogenic nature [48].

The existence of correlations between experimental data does not imply by itself a direct dependence or relationship between them, but clusters of tight correlations usually evidences some sort of interrelationship. In the present study, the considerable similitude of patterns of enzyme gene expression with that of a regulative factor such as SREBP1c, as well as its synchronism along the lipogenic pathway, hint at two plausible (albeit not excluding) explanations: (a) the effects of both OE and corticosterone on lipid metabolism are largely mediated through SREBP1c or/and (b) the changes in this regulative protein follow the same pattern induced overall on lipid metabolism by other factors so far unidentified. Since SREBP1c is a well known intermediary element in a signaling pathway that is affected both by insulin [30], sterols [31] and energy substrates themselves [49], it may be speculated that in any case there is a direct implication of SREBP1c in executing the combined signals of glucocorticoids and OE. But these effects are mediated and modulated by the different control environments of the tissues studied, which hints at other intermediaries placed between OE/corticosterone and SREBP1c, more than between the latter and the modulation of the expression of lipid metabolism enzyme genes.

Adrenalectomy is a severe insult to hormonal homeostasis, since it breaks up the HPA axis and removes a key element of control of energy, defense and humoral systems. Regardless of the loss of the adrenal medulla, corticosterone replacement may be insufficient to restore steroid hormone function, because the adrenal cortex secrete a number of other hormones, including androgen, DHEA, estrogen, etc. [50]. Nevertheless, corticosterone returns most of the systems to a certain degree of normalcy. Adrenalectomized rats not receiving the benefits of hormonal replacement pellets are in dire straits and need to fulfill somehow the lack of corticosteroids to maintain its hormone-related energy homeostasis. Under OE treatment, glucocorticoid signaling is enhanced by the increased activation of 11β-hydroxysteroid dehydrogenase expression (and activity) in liver induced by OE [36], which combined with lower plasma corticosterone binding [51] results in a higher fraction of free corticosteroid in serum. Nevertheless, it is plausible that other adrenal hormones may play a moderating effect on glucocorticoid action [52].

The large array of data studied indicates that interaction of OE and glucocorticoids is different depending on the organ studied, a difference that holds even when applied to different WAT sites. We cannot explain where their signals get intermixed, but SREBP1c remains a most promising intermediate. It is a key player for OE action in liver [27], but its response to combined OE and glucocorticoids was fairly different in the other organs studied, despite the clear relationship existing on the direction and extent of change of its expression with those of important energy and lipid handling genes.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jsbmb.2009.06.003.

References

[1] M.M. Grasa, C. Cabot, M. Esteve, P. Yubero, R.M. Masanés, M.T. Blay, R. Vil, J. López-Martí, J.A. Fernández-López, X. Remesar, M. Alemany, Daily oral oleoyl-estrone gavage induces a dose-dependent loss of fat in Wistar rats, Obesity Res. 9 (2001) 202–209.
[2] X. Remesar, J.A. Fernández-López, M.T. Blay, P. Savall, A. Salas, M. Díaz-Silva, M. Esteve, M.M. Grasa, M. Alemany, Effect of oral oleoyl-estrone on adipose tissue composition in male rats, Int. J. Obesity 26 (2002) 1092–1102.
[3] A. Salas, X. Remesar, M. Esteve, Oleoyl-estrone treatment activates apoptotic mechanisms in white adipose tissue, Life Sci. 80 (2007) 293–298.
[4] D. Sanchis, F. Balada, M.M. Grasa, J. Virgili, J. Peinado, C. Monserrat, J.A. Fernández-López, X. Remesar, M. Alemany, Oleoyl-estrone induces the loss of body fat in rats, Int. J. Obesity 20 (1996) 588–594.
[5] F. Balada, D. Sanchis, M.M. Grasa, J. Virgili, J. Estruch, J.A. Fernández-López, X. Remesar, M. Alemany, Effect of the slimming agent oleoyl-estrone in liposomes on the body weight of Zucker obese rats, Int. J. Obesity 21 (1997) 789–795.
[6] M.M. Grasa, R. Vilà, M. Esteve, C. Cabot, J.A. Fernández-López, X. Remesar, M. Alemany, Oleoyl-estrone lowers the body weight of both ob/ob and db/db mice, Horm. Metabol. Res. 32 (2000) 246–250.
[7] J. López-Martí, M. Díaz-Silva, A. Salas, M.M. Grasa, J.A. Fernández-López, X. Remesar, M. Alemany, Oleoyl-estrone induces the massive los of body weight in Zucker fa/fa rats fed a high-energy hyperlipidic diet, J. Nutr. Biochem. 11 (2000) 530–535.
[8] M.M. Romero, M. Esteve, M. Alemany, Combined effects of oral oleoyl-estrone and limited food intake on body composition of young overweight male rats, Int. J. Obesity 30 (2006) 1149–1156.
[9] X. Remesar, P. Guijarro, C. Torregrosa, M.M. Grasa, J. López, J.A. Fernández-López, M. Alemany, Oral oleoyl-estrone induces the rapid loss of body fat in Zucker lean rats fed a hyperlipidic diet, Int. J. Obesity 24 (2000) 1405–1412.
[10] M. Blay, J. Peinado-Onsurbe, M.M. Grasa, M. Díaz-Silva, J.A. Fernández-López, X. Remesar, M. Alemany, Effect of oral oleoyl-estrone treatment on plasma lipoproteins and tissue lipase activities of Zucker lean and obese female rats, Int. J. Obesity 26 (2002) 618–626.
[11] D. Sanchis, C. Adán, A. Ardévol, M.M. Grasa, C. Cabot, F. Balada, R. Vilà, J. Estruch, M. Puerta, J.A. Fernández-López, X. Remesar, M. Alemany, Short-term treatment with oleoyl-oestrone in liposomes (Merlin-2) strongly reduces the expression of the ob gene in young rats, Biochem. J. 326 (1997) 357–360.
[12] C. Cabot, M.M. Grasa, J. Estruch, J.A. Fernández-López, X. Remesar, M. Alemany, Zucker obese rats are insensitive to the CRH-increasing effect of oleoyl-estrone, Brain Res. Bull. 46 (1998) 529–534.
[13] M.M. Grasa, C. Cabot, C. Adán, R. Vilà, M. Esteve, J. Estruch, J.A. Fernández-López, X. Remesar, M. Alemany, Effect of adrenalectomy on the slimming activity of liposome-carried oleoyl-estrone in the rat, Int. J. Obesity 22 (1998) 1225–1230.
[14] M.M. Grasa, M. Serrano, J.A. Fernández-López, M. Alemany, Corticosterone inhibits the lipid-mobilizing effects of oleoyl-estrone in adrenalectomized rats, Endocrinology 148 (2007) 4056–4063.
[15] C.B. Whorwood, S.J. Donovan, D. Flanagan, D.I.W. Phillips, C.D. Byrne, Increased glucocorticoid receptor expression in human skeletal muscle cells may contribute to the pathogenesis of the metabolic syndrome, Diabetes 51 (2002) 1066–1075.
[16] Y.Z. Wei, K.M. Chen, A.T. Whaley-Connell, C.S. Stump, J.A. Ibdah, J.R. Sowers, Skeletal muscle insulin resistance: role of inflammatory cytokines and reactive oxygen species, Am. J. Physiol. 294 (2008) R673–R680.
[17] L. Mantha, E. Palacios, Y. Deshaies, Modulation of triglyceride metabolism by glucocorticoids in diet-induced obesity, Am. J. Physiol. 277 (1999) R455–R464.
[18] C.G. Jones, S.K. Hothi, M.A. Titheradge, Effect of dexamethasone on gluconeogenesis, pyruvate kinase, pyruvate carboxylase and pyruvate dehydrogenase flux in isolated hepatocytes, Biochem. J. 289 (1993) 821–828.
[19] J.J. Tomlinson, A. Boudreau, D.M. Wu, E. Atlas, R.J.G. Hache, Modulation of early human preadipocyte differentiation by glucocorticoids, Endocrinology 147 (2006) 5284–5293.
[20] K. Sun, L. Myatt, Enhancement of glucocorticoid-induced 11b-hydroxysteroid dehydrogenase type 1 expression by proinflammatory cytokines in cultured human amnion fibroblasts, Endocrinology 144 (2003) 5568–5577.
[21] C. Asensio, P. Muzzin, F. Rohner-Jeanrenaud, Role of glucocorticoids in the physiopathology of excessive fat deposition and insulin resistance, Int. J. Obesity 28 (Suppl. 4) (2004) S45–S52.
[22] M. Serrano-Muñoz, J.A. Fernández-López, M. Alemany, M.M. Grasa, Oleoyl-estrone increases the availability of glucocorticoids in liver but limits its effects on phosphoenolpyruvate carboxykinase, in: 12th International Congress on Hormonal Steroids and Hormones & Cancer, Athens, 2006, pp. 273–274, P125.
[23] M.M. Grasa, C. Cabot, J.A. Fernández-López, X. Remesar, M. Alemany, Modulation of corticosterone availability to white adipose tissue of lean and obese Zucker rats by corticosteroid-binding globulin, Horm. Metabol. Res. 33 (2001) 407–411.
[24] J. Ruzzin, A.S. Wagman, J. Jensen, Glucocorticoid-induced insulin resistance in skeletal muscles: defects in insulin signalling and the effects of a selective glycogen synthase kinase-3 inhibitor, Diabetologia 48 (2005) 2119–2130.
[25] N. Paquot, P. Schneiter, E. Jéquier, L. Tappy, Effects of glucocorticoids and sympathomimetic agents on basal and insulin-stimulated glucose metabolism, Clin. Physiol. 15 (1995) 231–240.
[26] G.X. Chen, G.S. Liang, J.F. Ou, J.L. Goldstein, M.S. Brown, Central role for liver X receptor in insulin-mediated activation of Srebp-1c transcription and stimulation of fatty acid synthesis in liver, Proc. Natl. Acad. Sci. U.S.A. 101 (2004) 11245–11250.
[27] M.M. Romero, J.A. Fernández-López, M. Alemany, M. Esteve, Gene expression modulation of liver energy metabolism by oleoyl-estrone in overweight rats. Biosci. Rep., in press. Published online 11/03/2009, doi:10.1042/BSR20080182.
[28] R. Raghow, C. Yellaturu, X. Deng, E.A. Park, M.B. Elam, SREBPs: the crossroads of physiological and pathological lipid homeostasis, Tr. Endocrinol. Metab. 19 (2008) 65–73.
[29] H. Shimano, SREBPs: physiology and pathophysiology of the SREBP family, FEBS J. 276 (2009) 616–621.
[30] M. Foretz, C. Guichard, P. Ferré, F. Foufelle, Sterol regulatory element binding protein-1c is a major mediator of insulin action on the hepatic expression of glucokinase and lipogenesis related genes, Proc. Natl. Acad. Sci. U.S.A. 96 (1999) 12737–21274.
[31] D.G. Yan, M. Lehto, L. Rasilainen, J. Metso, C. Ehnholm, S. Ylä-Herttuala, M. Jauhiainen, V.M. Olkkonen, Oxysterol binding protein induces upregulation of SREBP-1c and enhances hepatic lipogenesis, Arterioscl. Thromb. Vasc. Biol. 27 (2007) 1108–1114.
[32] M.T. Serafini, M. Alemany, A micromethod for the enzymatic estimation of the degree of ramification of glycogen, J. Biochem. Biophys. Methods 15 (1987) 33–39.
[33] M. Somogyi, Preparation of glycogen, nitrogen- and phosphorus-free, from liver, Methods Enzymol. 3 (1957) 3–4.
[34] J. Folch, M. Lees, G.H. Sloane-Stanley, A simple method for the isolation and purification of total lipides from animal tissues, J. Biol. Chem. 226 (1957) 497–509.
[35] M.M. Romero, M.M. Grasa, M. Esteve, J.A. Fernández-López, M. Alemany, Semi-quantitative RT-PCR measurement of gene expression in rat tissues including a correction for varying cell size and number, Nutr. Metab. 4 (2007) 26.

[36] M. Franco-Colín, A.M. Téllez-López, L. Quevedo-Corona, R. Racotta, Effects of long-term high-sucrose and dexamethasone on fat depots, liver fat, and lipid fuel fluxes through the retroperitoneal adipose tissue and splanchnic area in rats, Metabol. Clin. Exp. 49 (2000) 1289–1294.
[37] K. Arai, T. Soga, H. Ohata, A. Otagiri, T. Shibasaki, Effects of food restriction on peroxisome proliferator-activated receptor-g and glucocorticoid receptor signaling in adipose tissues of normal rats, Metabolism 53 (2004) 28–36.
[38] J.D. Horton, Y. Bashmakov, I. Shimomura, H. Shimano, Regulation of sterol regulatory element binding proteins in livers of fasted and refed mice, Proc. Natl. Acad. Sci. U.S.A. 95 (1998) 5987–5992.
[39] P. Seale, B. Bjork, W.L. Yang, S. Kajimura, S. Chin, S.H. Kuang, A. Scimè, S. Devarakonda, H.M. Conroe, H. Erdjument-Bromage, P. Tempst, M.A. Rudnicki, D.R. Beier, B.M. Spiegelman, PRDM16 controls a brown fat/skeletal muscle switch, Nature 454 (2008) 961–967.
[40] H.H. Shimano, Sterol regulatory element-binding protein family as global regulators of lipid synthetic genes in energy metabolism, Vitam. Horm. 65 (2002) 167–194.
[41] M.M. Romero, J.A. Fernández-López, M. Esteve, M. Alemany, Site-related white adipose tissue lipid-handling response to oleoyl-estrone treatment in overweight male rats. Eur. J. Nutr. 48, in press. Published online on 27/03/2009, doi:10.1007/s00394-009-0013-2.
[42] M.M. Romero, J.A. Fernández-López, M. Esteve, M. Alemany, Oleoyl-estrone inhibits lipogenic, but maintains thermogenic gene expression of brown adipose tissue in overweight rats, Biosci. Rep. 29 (2009) 237–243.
[43] M.M. Grasa, M. Esteve, R.M. Masanés, P. Yubero, M. Blay, J. López-Martí, C. Cabot, R. Vilà, J.A. Fernández-López, X. Remesar, M. Alemany, Oral gavage of oleoyl-estrone has a stronger effect on body weight in male obese Zucker rats than in female, Diabet. Obes. Metab. 3 (2001) 203–208.
[44] T.B. Walden, J.A. Timmons, P. Keller, J. Nedergaard, B. Cannon, Distinct expression of muscle-specific microRNAs (myomirs) in brown adipocytes, J. Cell. Physiol. 218 (2009) 444–449.
[45] D.O. Foster, F. Depocas, M.L. Frydman, Noradrenaline-induced calorigenesis in warm-acclimated and in cold-acclimated rats—relations between concentration of noradrenaline in arterial plasma, blood-flow to differently located masses of brown adipose tissue, and calorigenic response, Can. J. Physiol. Pharmacol. 58 (1980) 915–924.
[46] D. Sanchis, F. Balada, C. Picó, M.M. Grasa, J. Virgili, C. Farrerons, A. Palou, J.A. Fernández-López, X. Remesar, M. Alemany, Rats receiving the slimming agent oleoyl-estrone in liposomes (Merlin-2) decrease food intake but maintain thermogenesis, Arch. Physiol. Biochem. 105 (1997) 663–672.
[47] L. Abu-Elheiga, M.M. Matzuk, K.A.H. Abo-Hashema, S.J. Wakil, Continuous fatty acid oxidation and reduced fat storage in mice lacking acetyl-CoA carboxylase 2, Science 291 (2001) 2613–2616.
[48] A.M.B. Moir, V.A. Zammit, Rapid switch of hepatic fatty acid metabolism from oxidation to esterification during diurnal feeding of meal-fed rats correlates with changes in the properties of acetyl-CoA carboxylase, but not of carnitine palmitoyltransferase I, Biochem. J. 291 (1993) 241–246.
[49] J. Xu, M.T. Nakamura, H.P. Cho, S.D. Clarke, Sterol regulatory element binding protein-1 expression is suppressed by dietary polyunsaturated fatty acids. A mechanism for the coordinate suppression of lipogenic genes by polyunsaturated fats, J. Biol. Chem. 274 (2003) 23577–23583.
[50] W.D. Drucker, J.M. Blumberg, H.M. Gandy, R.R. David, A.L. Verde, Biologic activity of dehydroepiandrosterone sulfate in man, J. Clin. Endocrinol. Metab. 35 (1972) 48–54.
[51] M.M. Grasa, C. Cabot, F. Balada, J. Virgili, D. Sanchis, C. Monserrat, J.A. Fernández-López, X. Remesar, M. Alemany, Corticosterone binding to tissues of adrenalectomized lean and obese Zucker rats, Horm. Metab. Res. 30 (1998) 699–704.
[52] M. Kalimi, Y. Shafagoj, R. Loria, D. Padgett, W. Regelson, Anti-glucocorticoid effects of dehydroepiandrosterone (DHEA), Mol. Cell. Biochem. 131 (1994) 99–104.