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Rats treated with oleoyl-oestrone maintain glucidic homeostasis: comparisons with a pair-fed model

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To determine whether or not the weight (and fat) loss induced by oleoyl-oestrone treatment results only as a consequence of decreased food intake, we compared treated animals with a pair-fed model. To this end, Wistar female rats received daily oral gavages of 10 μmol/kg per d oleoyl-oestrone in sunflower oil, or vehicle alone for 10 or 20 d. A second group of rats received the gavage of sunflower oil and the same amount of food ingested as the oleoyl-oestrone-treated animals (pair-fed group). Rats treated with oleoyl-oestrone maintained glucidic metabolism homeostasis despite a marked decrease in adipose tissue weight ($P<0{\cdot}001$). Pair-fed rats exhibited a different pattern, comparable to short-term starvation, with greatly decreased glycogen stores ($P<0{\cdot}0001$). The most significant effects were detected in the 10 d period groups. Oleoyl-oestrone affected the activity of the ponderostat system not only by decreasing appetite but also by modifying energy partition: treated animals maintained their glucose and energy homeostasis despite decreased food intake and the massive depletion of lipid stores.

Oleoyl-oestrone: Body weight: Pair-fed

Oleoyl-oestrone (OE) is synthesized from oestrone by adipose cells (Esteve *et al.* 2001) and released into the bloodstream, where its concentrations correlate with body fat mass (Fernández-Real *et al.* 1999; Cabot *et al.* 2000). As its experimental administration results in the loss of body fat, without concurrent loss of body protein (Sanchis *et al.* 1996; Grasa *et al.* 2001), OE has been postulated as a lipostatic signal regulating body fat mass. The intravenous administration of pharmacological doses of OE causes mild oestrogenic effects, and results in high circulating levels of oestrone (Cabot *et al.* 2001). Oral administration of the oestrone ester, however, precludes these effects by maintaining low plasma levels of both oestrone and oestradiol (Cabot *et al.* 2001). The decrease in food intake induced by OE is very sharp in the first 2 d of treatment, but rapidly recovers thereafter; this contrasts with the pattern observed for body weight, which maintains a gap in relation to controls a long time after treatment has ceased (Adán *et al.* 1999).

Although the mechanism underlying OE action remains unknown, it may involve pathways other than those activated by starvation. Thus, chronic food restriction leads to a decrease in body weight, resulting from a marked increase in lipid mobilization (Comizio *et al.* 1998) that contrasts with the pattern seen in short-term starvation, with limited lipolysis and rapid utilization of glycogen stores, inducing a dramatic decrease in liver weight (Palou *et al.* 1981). The present study was designed to establish whether or not the effects of OE on the intermediate metabolism were simply a consequence of decreased food intake. Thus, we compared OE-treated female rats over a period of either 10 or 20 d with rats that were fed the same amount of food as that consumed by the treated rats (pair-fed, PF).

Materials and methods

Wistar female rats (Harlan-Interfauna Ibérica, Sant Feliu de Codines, Spain) weighing initially 220–230 g were housed in individual cages under a light cycle (on from 08.00 to 20.00 hours) and in a temperature-controlled environment (20–22°C). Food (standard rat chow pellets; Panlab, Barcelona, Spain) and water were provided in excess at all times, except for PF groups. Food consumption was measured daily and used to compute the mean energy intake of the animals in each group based on the energy content of the rat chow (digestible energy: 13·26 MJ/kg).

All procedures were in accordance with the guidelines for the use of experimental animals established by the European Union, Spain and Catalonia, and were approved by the Animal Handling Ethics Committee of the University of Barcelona.

Rats were randomly divided into two groups: one group was treated for 10 d and the other for 20 d. Each group was further sub-divided into three groups (six rats per group) depending on treatment: one group received a daily intra-gastric gavage of 0·2 ml sunflower oil containing 10 μmol OE (OED SL, Barcelona, Spain) per kg rat weight (OE-treated group); the other two groups received only the intra-gastric gavages of sunflower oil, the first fed *ad libitum* (control group), the second having its food access limited to amounts consumed by the OE-treated animals (PF group).

On day 10 (or 20), and at the beginning of the light cycle, rats were killed by decapitation and blood was recovered in plastic beakers. Serum was separated and frozen for analysis. The liver,

Abbreviations: OE, oleoyl-oestrone; PF, pair-fed; WAT, white adipose tissue.

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pieces of white adipose tissue (WAT) from different locations (periovaric, retroperitoneal), interescapular brown adipose tissue and gastrocnemius, tibialis, soleus and extensorum digitorum longus muscles were immediately excised, frozen in liquid N and stored at −80°C. All tissue samples were weighed before use.

Lipid content of liver and gastrocnemius muscle samples were extracted with trichloromethane–methanol (2:1), dried and subjected to direct weighing (Folch *et al.* 1957). Tissue samples were used for glycogen determination as glycosyl residues (Serafini & Alemany, 1987). DNA content was measured in WAT samples using a standard fluorimetric method with 3,5-diaminobenzoic acid (Sigma Chemical Co., St Louis, MO, USA) and bovine thymus DNA as a standard (Remesar *et al.* 2002). Since all cell nuclei contain the same amount of DNA (about 6 μg per million cells), we could estimate the approximate number of cells in a given WAT site by dividing its DNA content by the mean DNA content of a cell (6 pg/cell). The mean mass of the cells in a given WAT site was determined by dividing the weight of the tissue by the number of cells it contained (Her *et al.* 1973; Remesar *et al.* 2002).

Serum was used to measure glucose (kit from Sigma), triacylglycerols (kit from Biosystems, Barcelona, Spain), NEFA (kit from Wako, Richmond, VA, USA), 3-hydroxybutyrate (kit from Roche Diagnostics, Mannheim, Germany), total cholesterol (kit from Menarini, Florence, Italy), HDL-cholesterol (kits from Randox, Crumlin, UK and from Menarini), insulin (sensitive rat insulin RIA kit from Linco, St Louis, MO, USA), leptin (rat insulin RIA kit from Linco), acyl-oestrone (Ardévol *et al.* 1997; Estrone RIA; DSL, Webster, TX, USA) and adiponectin (mouse adiponectin kit from Linco).

Prism 4 program was utilized for statistical analysis (GraphPad Software, San Diego, CA, USA). Two-way ANOVA was employed either to compare all experimental groups or to compare only the OE and PF groups. *Post hoc* Bonferroni tests were used to establish the differences between groups. The α level for *post hoc* comparisons was 0·95.

Results

Rats treated with OE exhibited markedly decreased food intake during the first 4 d of treatment (Fig. 1); they subsequently recovered to control levels on day 10. OE-treated animals ate a mean of 163 (SEM 8·4) g over 10 d compared with 196 (SEM 7·11) g consumed by control rats. The intake differences between OE and PF groups *v.* control group were significant only during the first 5 d of treatment. Between days 10 and 20 the control group consumed 193 (SEM 8·11) g while the OE and PF groups ate 169 (SEM 11·1) g.

Fig. 2 displays the changes in body weight during the period studied. The OE group exhibited significantly lower body weight than the PF group during treatment. Thus, there is an initial decrease in body weight in both the OE and PF groups, with the lowest values occurring on day 4, followed by a partial recovery running parallel with the control group; however, this gap is maintained until the end of the study. It should be noted that the recovery for the PF group tended to be faster than that of OE-treated rats.

Table 1 demonstrates that the serum levels of glucose, NEFA, 3-hydroxybutyrate and triacylglycerols were not affected by OE, regardless of the experiment's duration. The control and OE groups differed only in total cholesterol and HDL-cholesterol

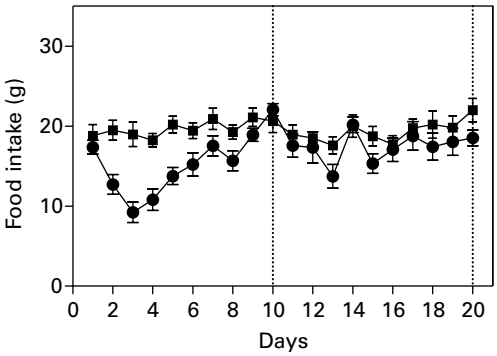

Fig. 1. Changes in food intake of Wistar female rats after 10 or 20 d of daily gavages of sunflower oil (control group, ■) or 10 μmol/kg oleoyl-oestrone (OE)/pair-fed (PF) group (●). PF rats were fed the same amount of food as the OE-treated group. For details of procedures, see p. 738. Values are the means (of six to twelve different animals) with their standard errors depicted by vertical bars. Significance of the differences between groups (two-way ANOVA): effect of time $P<0·0001$; effect of treatment $P<0·0001$. Bonferroni post-test: control and OE-treated values were different on days 2 and 5 ($P<0·05$), as well as on days 3 and 4 ($P<0·001$). There were no differences between OE-treated and PF groups.

levels, proving particularly low in the OE group; conversely, the PF group showed significantly increased levels of lipid metabolites on day 10, although this was not maintained on day 20, when only a decrease in glucose and urea and an increase in 3-hydroxybutyrate were recorded. Direct comparison of the OE and PF groups uncovered significant differences in urea, triacylglycerols, NEFA, 3-hydroxybutyrate, cholesterol and HDL-cholesterol.

Serum hormone levels revealed important differences between treated groups and controls, as can be seen in Table 2. On day 10, OE-treated rats exhibited lower insulin and leptin levels, and higher acyl-oestrone levels than did controls, these differences remaining intact on day 20 in the case of leptin. The PF group displayed lower leptin and insulin and higher adiponectin levels on day 10 than did controls, the differences in leptin and insulin

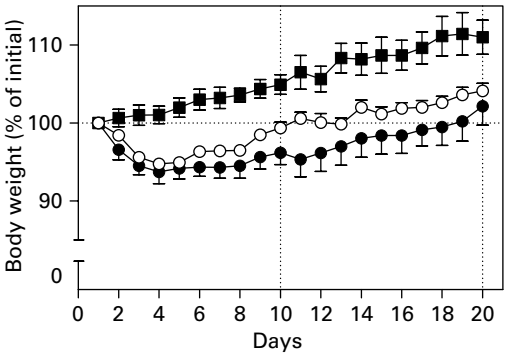

Fig. 2. Changes in body weight, expressed as the percentage of initial body weight, of Wistar female rats after 10 or 20 d of daily gavages of sunflower oil (control group, ■) or 10 μmol/kg oleoyl-oestrone (OE) (●) and the pair-fed (PF) group (○). For details of procedures, see p. 738. Values are the means (of six to twelve different animals) with their standard errors depicted by vertical bars. Significance of the differences between groups (two-way ANOVA): effect of time $P<0·0001$; effect of treatment $P<0·0001$. Bonferroni post-test: control and OE-treated values were different from day 3 of treatment ($P<0·05$). Two-way ANOVA restricted to OE-treated and PF groups showed significant effects on both time ($P<0·0001$) and treatment ($P<0·0003$), without any interaction ($P=0·9056$) or differences at any point when the Bonferroni test was applied.

Table 1. Serum metabolite levels of Wistar female rats after 10 or 20 d of treatment*

(Mean values with their standard errors for six animals per group)

	10 d						20 d						*P*†		
	Control		OE-treated		PF		Control		OE-treated		PF				
Parameter	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Time	Treatment	OE *v.* PF
Glucose (mM)	5·74^{a}	0·08	5·55^{a}	0·11	5·68^{a}	0·13	6·17^{a}	0·30	5·71^{a}	0·10	5·29^{b}	0·10	0·6221	0·0209	0·1291
Urea (mM)	5·00^{a}	0·38	5·50^{a}	0·49	4·67^{a}	0·26	5·99^{a}	0·47	6·64^{a}	0·27	4·93^{b}	0·56	0·0282	0·0193	0·0067
Triacylglycerols (mM)	0·40^{a}	0·04	0·46^{a}	0·03	0·73^{b}	0·08	0·61^{a}	0·09	0·46^{a}	0·05	0·56^{a}	0·06	0·8083	0·0180	0·0047
NEFA (mM)	0·38^{a}	0·05	0·43^{a}	0·03	0·65^{b}	0·01	0·49^{a}	0·05	0·38^{a}	0·02	0·50^{a}	0·05	0·1856	0·0002	0·0002
3-Hydroxybutyrate (μM)	100^{a}	9·01	104^{a}	15	193^{b}	17	82·1^{a}	4·11	104^{a}	10·1	175^{b}	9·02	0·3566	<0·0001	0·0004
Total cholesterol (mM)	1·42^{a}	0·11	0·69^{b}	0·12	1·30^{a}	0·08	1·21^{a}	0·03	0·68^{b}	0·11	1·19^{a}	0·08	0·1638	<0·0001	<0·0001
HDL-cholesterol (mM)	1·29^{a}	0·05	0·38^{b}	0·06	1·18^{a}	0·09	0·82^{a}	0·05	0·48^{b}	0·09	0·83^{a}	0·07	0·0004	<0·0001	<0·0001

OE, oleoyl-oestrone; PF, pair-fed.

^{a,b}Mean values within a row with unlike superscript letters were significantly different (*post hoc* comparisons, Bonferroni test; $P<0·05$).

*For details of procedures, see p. 738.

†Significance of the differences between groups (ANOVA).

Table 2. Plasma hormone values of Wistar female rats after 10 or 20 d of treatment*

(Mean values with their standard errors for six animals per group)

	10 d						20 d						*P*†		
	Control		OE-treated		PF		Control		OE-treated		PF				
Parameter	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Time	Treatment	OE *v.* PF
Insulin (nM)	0·37^{a}	0·06	0·24^{b}	0·04	0·15^{b}	0·01	0·55^{a}	0·04	0·43^{b}	0·03	0·25^{b}	0·03	0·0002	0·0001	<0·0001
Leptin (nM)	0·16^{a}	0·02	0·08^{b}	0·01	0·09^{b}	0·02	0·22^{a}	0·02	0·11^{b}	0·01	0·11^{b}	0·02	0·0376	0·0001	0·6824
Adiponectin (nM)	144^{a}	12	109^{a}	14	229^{b}	28	182^{a}	21	161^{a}	14	193^{a}	13	0·2184	0·0005	0·0004
Acyl-oestrone (nM)	147^{a}	7·7	222^{b}	17	181^{ab}	21	206^{a}	5·7	187^{a}	21	196^{a}	16	0·3176	0·183	0·3840

OE, oleoyl-oestrone; PF, pair-fed.

^{a,b}Mean values within a row with unlike superscript letters were significantly different (*post hoc* comparisons, Bonferroni test; $P<0·05$).

*For details of procedures, see p. 738.

†Significance of the differences between groups (ANOVA).

still maintained at day 20. Direct comparison of the OE and PF groups indicated that insulin and adiponectin levels were, in fact, different.

Table 3 depicts the absolute and relative weights of liver and different muscles. In OE-treated rats the weights of the liver and soleus, tibialis and extensorum digitorum longus muscles remained the same, but exhibited a lower gastrocnemius muscle mass than controls, on both days 10 and 20. The PF group maintained the same muscle weight values but decreased values for liver, on both days 10 and 20. As a consequence, the relative weight of the liver in PF were lower than in the control group, contrasting with the increased values displayed by the OE group on day 20. Muscles of treated groups maintained their relative values in comparison with the control group, except for gastrocnemius muscle on day 10. Direct comparison between OE and PF groups indicated that liver, gastrocnemius, tibialis and extensorum digitorum longus muscle weights were different.

Table 4 displays the absolute and relative weights of adipose tissue sites, as well as the mean cell weight and number. The OE group showed lower adipose tissue weights, significantly so both in the case of periovaric location on day 10, and for all adipose samples on day 20. This same pattern was repeated by the relative weight of tissues. The number of cells decreased in periovaric and retroperitoneal adipose tissue with OE treatment, on both days 10 and 20 of treatment. PF groups revealed a tendency toward lower adipose tissue weights, the differences being significant for retroperitoneal (due to decreased cell numbers) on day 10, as well as for all adipose samples on day 20. Direct comparison between OE and PF groups indicated that periovaric WAT weight and cell number were also different between these groups. Brown adipose tissue showed significant decreases for the PF group on days 10 and 20, whereas in the OE group this decrease was significant only on day 20.

Table 5 displays values for liver and gastrocnemius muscle lipid, protein and glycogen content. Treatment with OE did not induce changes in liver and muscle metabolite content, neither on day 10 nor day 20, except for a small increase in protein content on day 20. However, the PF group exhibited an almost complete depletion of glycogen levels, particularly on day 10, the decrease being more marked in liver than in muscle. There were significant differences in liver protein, as well as in liver and muscle glycogen content between the OE and PF groups.

Discussion

Control rats exhibited normal growth patterns during treatment, since the extra energy provided by oil gavages represented only 3 % (8·6 kJ/d) of the energy derived from rat chow (265 kJ/d). Serum hormone and metabolite levels were similar to those previously described (Grasa *et al.* 2001), with the decrease in cholesterol and HDL-cholesterol levels proving the most remarkable occurrence. The detected decreases in total cholesterol were as pronounced as previously described, being the important decrease in HDL fraction partially counterbalanced by the increased cholesterol content of other lipoproteins, mainly in the VLDL fraction (Blay *et al.* 2002).

The response of OE-treated animals in terms of body weight and food intake was the same as that described for a 10 d treatment (Grasa *et al.* 2001), implying a rapid recovery of food intake following a short period of restricted intake (about 4 d), which is not counterbalanced by a recovery in body weight. This sequence of events had two consecutive periods: the initial 10 d, where food restriction in the OE and PF groups represented a 17 % decrease in the energy ingested by controls (predominantly during the first half of the period); and a second period, wherein food restriction represented only a 13 % decrease in the energy ingested by controls. It might therefore be expected that the effects of energy intake limitation should be more intense in the groups treated for 10 d than in those treated for 20 d. However, the effects induced by treatment or dietary restriction in the 10 d groups seriously affected recovery, since the partial increase in food intake between days 10 and 20 was not followed by a rapid weight recovery. As the slopes in the growth pattern were similar from day 4 onwards, the differences in body weight were maintained between treated and control groups. Loss of adipose tissue mass was the major contributing factor to weight loss, consistent with previous studies (Grasa *et al.* 2001). This is also in accordance with the role postulated for OE in modifying the ponderostat setting (Adán *et al.* 1999).

We can assume that the decrease in body weight in OE-treated rats ultimately resulted from the negative energy balance stemming from the unchanged energy expenditure (Sanchis *et al.* 1997*b*) combined with a decreased energy intake. This imbalance derived principally from lipid mobilization, as confirmed by adipose tissue depletions from various locations during the first half of the period. In fact, this loss was maintained during the second 10 d, despite the resumption of relatively normal food intake. Lipid mobilization persisted while maintaining normal glucose and glycogen levels, indicating that the body's response to the energy imbalance was highly selective, i.e. the retention of carbohydrate stores coupled with the mobilization of lipid resources. The tendency to increase liver glycogen levels in the OE group on day 20 is consistent with our previous reports (Sanchis *et al.* 1997*a*), and explains the increase in relative weight.

The decrease in adipose tissue mass in OE-treated animals was caused by a corresponding decrease in cell numbers, consistent with a predominance of apoptotic mechanisms (Troyer & Fernandes, 1996). The present results are partially in accordance with previously reported gender-specific patterns (Porter *et al.* 2004) in rats with restricted energy intake, specifically the tendency of females to maintain adipocyte volume, despite the varying sensitivities of adipose locations.

The PF group followed a different growth recovery pattern than the OE-treated group, involving more pronounced changes in liver weight (resulting from a near total depletion of glycogen and its associated water) and more pronounced than those in adipose tissue, especially during the first 10 d. This pattern continued over the second 10 d period, since low liver weight and hepatic glycogen were maintained. The scarcely detectable mobilization of adipose tissue during the first 10 d, when only the retroperitoneal location showed a significant weight loss despite the decreased cell numbers on day 20, contrasts with the pattern of OE-treated rats, and may, in fact, be consistent with the increased lipolysis observed in these groups (Grasa *et al.* 2001). The metabolic pattern followed by the PF group was similar to that induced by short-term starvation, with lower glucose levels and a dramatic decrease in liver and muscle glycogen, together with enhanced markers for lipolytic activity, i.e. the increase in NEFA and ketone bodies. Such decreases in both leptin and insulin levels support this interpretation (Rabinovitch *et al.* 1976; Baranowska *et al.* 2001), as do the increases in adiponectin levels (Zhang *et al.* 2002). Since over the last 4 d these rats ingested nearly 90 % of the control intake, it

Table 3. Liver and muscle tissue weight and tissue relative weight (as a percentage of body weight (%bw)) in Wistar female rats after 10 or 20 d of treatment*

(Mean values with their standard errors for six animals per group)

		10 d						20 d						*P*†		
		Control		OE-treated		PF		Control		OE-treated		PF				
Parameter	Units	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Time	Treatment	OE *v.* PF
Liver	g	9·94[a]	0·25	9·02[a]	0·28	6·68[b]	0·28	9·55[a]	0·32	10·1[a]	0·25	7·91[b]	0·38	0·0013	< 0·0001	< 0·0001
	%bw	3·88[a]	0·11	4·03[a]	0·16	2·96[b]	0·02	3·74[a]	0·10	4·52[b]	0·09	3·30[b]	0·10	0·0080	< 0·0001	< 0·0001
Gastrocnemius	g	1·43[a]	0·08	1·19[c]	0·06	1·32[a]	0·08	1·54[a]	0·10	1·34[b]	0·09	1·51[a]	0·09	0·0006	0·0773	0·0059
	%bw	0·59[a]	0·02	0·52[b]	0·02	0·59[a]	0·02	0·60[a]	0·01	0·57[a]	0·01	0·63[a]	0·02	0·0042	0·0048	0·0051
Soleus	mg	101[a]	9	96[a]	9	104[a]	11	124[a]	10	127[a]	10	116[a]	9	0·0017	0·9594	0·8505
	%bw	0·042[a]	0·001	0·043[a]	0·003	0·046[a]	0·002	0·049[a]	0·002	0·055[a]	0·009	0·049[a]	0·002	0·0287	0·6309	0·7486
Extensorum	mg	107[a]	9	101[a]	10	112[a]	8	125[a]	10	113[a]	10	130[a]	11	0·0017	0·0441	0·0045
digitorum longus	%bw	0·044[a]	0·002	0·041[a]	0·006	0·050[a]	0·001	0·049[a]	0·003	0·048[a]	0·002	0·054[a]	0·003	0·0345	0·0249	0·0144
Tibialis	mg	468[a]	12	421[a]	14	457[a]	13	511[a]	18	445[a]	15	512[a]	7	0·0259	0·0203	0·0386
	%bw	0·19[a]	0·004	0·18[a]	0·02	0·20[a]	0·005	0·20[a]	0·005	0·19[a]	0·007	0·21[a]	0·005	0·3756	0·0934	0·0691

OE, oleoyl-oestrone; PF, pair-fed.

[a,b]Mean values within a row with unlike superscript letters were significantly different (*post hoc* comparisons, Bonferroni test; $P < 0·05$).

*For details of procedures, see p. 738.

†Significance of the differences between groups (ANOVA).

Table 4. White adipose tissue (WAT) weight, tissue relative weight (as % of body weight (%bw)), cell numbers and cell mass in Wistar female rats after 10 or 20 d of treatment*

(Mean values with their standard errors for six animals per group)

		10 d						20 d						*P*†		
		Control		OE-treated		PF		Control		OE-treated		PF				
Parameter	Units	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Time	Treatment	OE *v.* PF
Retroperitoneal WAT	g	1·73[a]	0·18	1·25[a]	0·21	1·37[a]	0·16	2·52[a]	0·21	1·35[b]	0·15	1·56[b]	0·15	0·0265	0·0003	0·3744
	%bw	0·78[a]	0·078	0·55[a]	0·086	0·61[a]	0·077	0·92[a]	0·119	0·73[b]	0·076	0·68[b]	0·075	0·0317	0·0003	0·1226
Cell number	$\times 10^6$	144[a]	16·5	83·8[b]	10·2	97·6[b]	12·9	143[a]	10·0	79·1[b]	12·1	67·1[b]	13·4	0·3585	0·0001	0·9281
Cell mass	ng	12·7[a]	1·10	15·2[a]	1·92	15·4[a]	1·89	19·3[a]	2·04	18·3[a]	2·67	19·9[a]	2·37	0·0089	0·7215	0·6903
Periovaric WAT	g	4·69[a]	0·25	2·74[b]	0·21	3·54[a]	0·35	5·71[a]	0·45	3·02[b]	0·22	3·99[b]	0·33	0·0916	<0·0001	0·0242
	%bw	1·95[a]	0·117	1·21[b]	0·130	1·49[a]	0·147	2·23[a]	0·227	1·29[b]	0·125	1·58[b]	0·257	0·2431	<0·0001	0·0606
Cell number	$\times 10^6$	328[a]	25·8	199[b]	11·8	372[a]	37·1	378[a]	65·8	206[b]	15·4	228[b]	16·6	0·3010	0·0005	0·0003
Cell mass	ng	14·9[a]	1·46	12·7[a]	1·78	9·85[a]	1·26	16·3[a]	1·66	14·8[a]	0·91	16·2[a]	1·78	0·0116	0·2611	0·6268
Interescapular brown	mg	365[a]	15	308[ab]	20	264[b]	20	401[a]	15	307[b]	22	327[b]	16	0·0782	0·0008	0·6179
adipose tissue	%bw	0·151[a]	0·006	0·136[a]	0·008	0·118[b]	0·007	0·157[a]	0·010	0·130[a]	0·011	0·136[a]	0·012	0·3175	0·0079	0·5135

OE, oleoyl-oestrone; PF, pair-fed.

[a,b]Mean values within a row with unlike superscript letters were significantly different (*post hoc* comparisons, Bonferroni test; $P<0·05$).

*For details of procedures, see p. 738.

†Significance of the differences between groups (ANOVA).

Table 5. Liver and gastrocnemius muscle metabolite content in Wistar female rats after 10 or 20 d of treatment*
(Mean values with their standard errors for six animals per group)

	10 d						20 d						*P*†		
	Control		OE-treated		PF		Control		OE-treated		PF				
Parameter	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Time	Treatment	OE *v.* PF
Liver glycogen (mg)	485[a]	39·1	352[a]	50·7	5·94[b]	1·71	518[a]	64·1	552[a]	45·4	127[b]	36·1	0·0055	0·0001	< 0·0001
Liver lipid (mg)	332[a]	19	308[a]	23	296[a]	13	274[a]	30	275[a]	32	11[a]	303	0·1228	0·8773	0·7295
Liver protein (g)	1·77[a]	0·13	1·64[ab]	0·08	1·43[b]	0·09	1·53[a]	0·11	1·89[b]	0·11	1·66[ab]	0·21	0·2937	0·0837	0·0091
Muscle glycogen (mg)	3·06[a]	0·27	3·21[a]	0·44	1·42[b]	0·34	3·93[a]	0·63	3·43[a]	0·61	2·60[a]	0·78	0·0868	0·0150	0·0225
Muscle lipid (mg)	22·1[a]	2·11	16·3[a]	2·1	19·8[a]	2·1	24·5[a]	5·1	19·8[a]	1·3	19·0[a]	2·2	0·3098	0·0226	0·4098
Muscle protein (mg)	261[a]	11	242[a]	18	237[a]	23	306[a]	31	257[a]	13	272[a]	21	0·0148	0·0654	0·7461

OE, oleoyl-oestrone; PF, pair-fed.
[a,b]Mean values within a row with unlike superscript letters were significantly different (*post hoc* comparisons, Bonferroni test; $P<0·05$).
*For details of procedures, see p. 738.
†Significance of the differences between groups (ANOVA).

remains difficult to attribute such dramatic changes to a simple trial of food deprivation, and a possible role of stress arising from the limitations of available food, as an intrinsic factor of a PF model, must be considered (Belda *et al.* 2005).

The restriction of food intake does not affect protein metabolism in the same way in the OE and PF groups. In OE-treated rats the stable urea levels, the maintenance (or increase on day 20) of liver protein content and the lack of relative weight changes in nearly all muscles indicate that protein was preserved and confirms the stability of this tissue, as has been previously reported (Cabot *et al.* 2000). However, the decrease of gastrocnemius muscle weight, perhaps as a consequence of slight decreases in lipid and protein contents, begs the question of whether different metabolic responses are the products of diverse fibre-type composition. The effects on the PF group are consistent with a possible loss of protein mass, similar to mechanisms underlying short-term starvation, particularly generated by liver protein, as is evident by the decrease in urea levels.

Decreases in insulin and leptin levels are not only typical of OE treatment (Adán *et al.* 1999), but are also consistent with the maintenance of energy balance at the expense of internal stores within the context of maintained internal homeostasis. However, the patterns followed by the OE-treated and PF groups, despite certain similar strategies regarding energy homeostasis, differ notably in the way glucose homeostasis was maintained: depleting glycogen in PF and decreasing its utilization in OE-treated rats. The more pronounced changes in insulin levels in PF groups, together with the increase in adiponectin levels on day 10, reinforce the different strategies employed by the OE and PF groups in managing their glucidic reserves. Moreover, the lipolytic pattern followed by the PF and OE groups is different, since the WAT weight decrease induced by OE is more pronounced than that caused by food restriction. Thus, the OE group does not display changes in plasma lipolytic markers, such as NEFA and 3-hydroxybutyrate levels, conversely to increased levels shown by the PF group, which are probably caused by an increase in hormone-sensitive lipase activity as in fast-state setting (Koopman *et al.* 1989). This fact allows us to suggest an accelerated utilization of these metabolites in the OE group that could explain the maintenance of glucidic stores.

Taken as a whole, the present findings confirm that lipid mobilization in OE-treated rats is not merely a consequence of food intake. We would furthermore suggest that treatment with OE induces certain selective changes in the control of sympathetic activity that probably induce a selective lipid mobilization without any change to glucidic homeostasis.

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