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Effects of oleoyl-estrone with dexfenfluramine, sibutramine or phentermine on overweight rats

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Abstract

We studied the combination of oleoyl-estrone with either dexfenfluramine, sibutramine or phentermine in overweight male rats treated for 10 days in order to determine whether they shared a mechanism of action. Oleoyl-estrone, dexfenfluramine and sibutramine decreased body weight and energy (essentially lipids); losses were higher when combined with oleoyl-estrone. Glycemia was maintained except under phentermine; oleoyl-estrone induced decreases in triacylglycerols, cholesterol, insulin and HOMA (homeostasis model assessment). Combination of oleoyl-estrone and sibutramine resulted in the loss of up to 29% body energy in 10 days. Energy expenditure was maintained. The effects of oleoyl-estrone and dexfenfluramine or sibutramine on appetite were substantially additive. All oleoyl-estrone-treated rats showed increased insulin sensitivity. In conclusion, combined treatment of overweight rats with oleoyl-estrone and sibutramine or dexfenfluramine results in a dramatic loss of weight and fat, whilst maintaining circulating energy homoeostasis.

Keywords: Obesity; Overweight; Energy balance; Oleoyl-estrone; Dexfenfluramine; Sibutramine; Phentermine

1. Introduction

Oleoyl-estrone decreases the body weight of normal-weight (Grasa et al., 2001a) and obese rats (Grasa et al., 2001b) or obese humans (Alemany et al., 2003), by inducing adipose tissue wasting (Remesar et al., 2002); it maintains glucose homoeostasis by decreasing insulin resistance (Grasa et al., 2001a) and favouring the alternative use of fat from internal stores as fuel for metabolic activity (Sanchis et al., 1990). Oleoyl-estrone also lowers the ponderostat setting (Adán et al., 1999), decreases food intake and maintains energy expenditure, thus creating a energy gap fulfilled by the mobilisation of the fat depots (Sanchis et al., 1990).

In conjunction with diet, dexfenfluramine induced the loss of excess weight in humans (Finer et al., 1987) and rodents (Beynen et al., 1986). Its main metabolic effect was the significant decrease in appetite (Blundell and Hill, 1992), elicited by its inhibition of serotonin reuptake in the brain (Turner, 1990). The association of dexfenfluramine with a thermogenic drug resulted in the enhancement of their separate slimming effectiveness (Wellman and Maher, 1999), but also surfaced a number of unwanted secondary effects of dexfenfluramine (Sachdev et al., 2002; Rich et al., 2003) that resulted in its removal from the market. However, the concept of association of drugs acting on different components of the equation of energy equilibrium, favouring the wasting of reserves may constitute a significant step in the development of pharmacological strategies for the treatment of obesity (Fernández-López et al., 2002).

Sibutramine (Bray and Greenway, 1999) is one of the few anti-obesity drugs now available in the market. It is widely used for the treatment of human overweight and non severe obesity (Ryan, 2004) as a complement of hypocaloric diets. Sibutramine acts inhibiting the synaptic reuptake of both serotonin and noradrenaline (Heal et al., 1998).

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Sibutramine decreases food intake and enhances energy expenditure (Hansen et al., 1999) through modulation of the efferent signals from the brain (Finer, 2002).

Phentermine is widely used as thermogenic drug (Arch, 1981), since it increases the availability of noradrenaline (Zychlinski and Montgomery, 1984), at least in part by inhibiting its reuptake (Samanin and Garattini, 1993). Its pharmacological effects decreasing body fat are limited (Mancini, 2003) and has been used in conjunction with other antiobesity drugs (Wellman and Maher, 1999) despite its adrenergic secondary effects (Jollis et al., 2000).

Oleoyl-estrone mobilises body fat, decreasing adipose tissue cellularity and cell size (Remesar et al., 2002); in addition, it decreases food intake. Since the precise mechanism of its modulation of appetite is unknown, we have studied the combination of oleoyl-estrone with either dexfenfluramine, sibutramine or phentermine in order to (a) determine whether the effects on food intake of oleoyl-estrone and the drugs acting on the food intake-controlling serotonin/noradrenaline pathways are additive or superimposable (i.e., they share totally or partially a mechanism of action) and (b) whether the combined administration of oleoyl-estrone and these anti-obesity drugs increases their effectiveness for the mobilisation of body reserves.

2. Materials and methods

Male Wistar rats (Harlan-Interfauna, Sant Feliu de Codines, Spain) of 45 days were used; they weighed initially 190–220 g. The rats were maintained in a controlled environment: 21.5–22.5 °C; 80% relative humidity; lights on from 08:00 to 20:00; they were kept in collective cages and were fed for 5 weeks a reduced cafeteria diet (Balada et al., 1997) ad libitum. At the end of this phase, the animals were already overweight. The rats were re-conditioned during an additional week with standard rat chow ad libitum (maintenance chow, Panlab, Barcelona, Spain) as sole food. They were used in the ensuing experiment at this point, when their age was 90 days.

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.

All animals received a daily gavage of 0.2 mL sunflower oil at the beginning of the light cycle and were maintained under standard conditions with full access to food pellets; their weights and food consumption were recorded daily. Eight groups of six animals were randomly selected: (a) controls; (b) oleoyl-estrone OE; (c) dexfenfluramine; (d) dexfenfluramine and oleoyl-estrone; (e) sibutramine; (f) sibutramine and oleoyl-estrone; (g) phentermine; and (h) phentermine and oleoyl-estrone. The gavage of rats in the control group contained only oil. The rats in the groups b, d, f and h were given a daily gavage containing oleoyl-estrone (OED, Barcelona, Spain), at a dose of 10 μmol/kg. Immediately after the oil gavage, groups c and d received a second gavage of 0.2 mL of a suspension of dexfenfluramine (Pharma Chem Lansheng Corp., Shanghai, China) in water at a daily dose of 3.0 mg/kg; groups e and f received a second gavage of 0.2 mL of a suspension of sibutramine (Pharma Chem Lansheng Corp.) in water at a daily dose of 5.0 mg/kg; and groups g and h received a second gavage of 0.2 mL of a suspension of phentermine (Sigma, St. Louis, MO, USA) in water at a daily dose of 5.0 mg/kg.

The treatments continued for 10 days. At the end of the experiment, the rats were killed by decapitation. Blood was received in dry beakers and allowed to clot. The serum was stored at −80 °C until processed. The rats were dissected, and the stomach and intestinal contents were removed; the carcass and organs (including the unused blood and packed blood cells) were sealed in polyethylene bags, autoclaved, and thoroughly homogenised (Grasa et al., 2001a). The rat paste was used for the estimation of lipid (Folch et al., 1957), and energy content, using a bomb calorimeter (C-7000 Ika, Heitersheim, Germany). Paste composition was related to in vivo weight correcting by digestive canal contents. The percentage body composition of controls was used to estimate the absolute lipid and energy content of the rats at the beginning of the experiment by applying these values to their known initial weights. The measured body weight and composition of the rats at the end of the study were used to determine the changes in body size and composition occurred during the 10 days of treatment.

Daily food intake in each cage was measured, and the mean food consumption of individual rats was estimated. Energy intake was calculated from the energy content of the food pellets and food intake. Rat chow had a mean energy content (bomb calorimeter) of 16.37 ± 0.04 kJ/g; this translated into a mean metabolisable energy of 13.3 kJ/g when discounting non-metabolisable fibre and assuming 95% efficiency in nutrient assimilation. Energy accrual was the difference between estimated energy on day 0 and the measured energy content (bomb calorimeter) on day 10. Mean energy expenditure was estimated as the difference between energy intake and net energy accrual.

Blood serum was used for the measurement of glucose (Trinder kit, Sigma, St. Louis, MO, USA), non-esterified fatty acids (NEFA kit, Wako Chemicals, Neuss, Germany), 3-hydroxybutyrate (kit 907979, Roche, Mannheim, Germany), total triacylglycerols (kit 11528, Biosystems, Barcelona, Spain), total cholesterol (Cholesterol reagent easy, Menarini, Firenze Italy), aspartate transaminase (Infinity AST reagent 51-25, Sigma Diagnostics, St. Louis, MO, USA) and alanine transaminase (Infinity ALT reagent 52-25, Sigma Diagnostics), as well as insulin (SRI-13 K, Linco, St. Charles, MO, USA). The HOMA (homeostasis model assessment) score (Matthews et al., 1985; Bonora et al., 2000) was calculated from the insulin and glucose data for each rat.

Statistical comparisons between groups were established by two-way analysis of variance programs.

3. Results

Fig. 1 shows the changes in body weight of the rats treated with oleoyl-estrone and/or dexfenfluramine, sibutramine or phentermine. The initial weights in the different experimental groups were controls 364 ± 14 g, oleoyl-estrone 356 ± 10 g, dexfenfluramine 360 ± 8 g, dexfenfluramine+oleoyl-estrone 360 ± 13 g, sibutramine 373 ± 13 g, sibutramine+oleoyl-estrone 368 ± 16 g, phentermine 374 ± 12 g and phentermine+oleoyl-estrone 374 ± 11 g. Controls increased slightly their weight in 10 days at a rate of less than 0.2% per day. The rats receiving dexfenfluramine or sibutramine lost weight at a rate lower than 0.4% per day; phentermine-treated rats did not lose weight at all. Oleoyl-estrone resulted in losses of about 0.8% per day, a value comparable to that obtained with the phentermine plus oleoyl-estrone treatment; the combination of oleoyl-estrone with sibutramine further accelerated the loss to 1.0% per day, and the combination with dexfenfluramine resulted in a loss of 1.1% per day. The effect of oleoyl-estrone on body weight was statistically significant, and the association with dexfenfluramine or sibutramine was also significantly different from the effects of these drugs alone; phentermine did not change body weight, but in combination with oleoyl-estrone the loss of body weight was comparable to that of oleoyl-estrone alone.

The analysis of body energy content is shown in Table 1. The energy content of the different groups followed the pattern outlined for body weight. The loss of lipid was in the range of 17–29% in all treated groups (except in the phentermine group) compared with controls: the sibutramine+oleoyl-estrone rats lost 29% of its lipid in just 10 days. Food intake decreased in all treated groups except for the phentermine group, maximally in the rats receiving oleoyl-estrone and either dexfenfluramine or sibutramine (i.e., a decrease of 43–47% in food intake vs. controls). No differences in the behaviour of the rats from the groups studied were observed, except for altered food intake.

Fig. 2 presents the energy balances of all groups. There were no significant differences in energy expenditure between all the groups. Since there was a lower energy intake in the sibutramine, dexfenfluramine, and all oleoyl-estrone-treated rats, energy expenditure was maintained in these groups at the expense of internal energy sources. Lipids accounted for most of this internal energy (67–98%; P values were significant for the effect of oleoyl-estrone, dexfenfluramine and sibutramine, but not for phentermine); in contrast, the contribution of all other internal energy sources (i.e., protein or glycogen) were not significant for any of the compounds tested.

Except for a decrease induced by dexfenfluramine, no significant effects were observed in the glucose levels

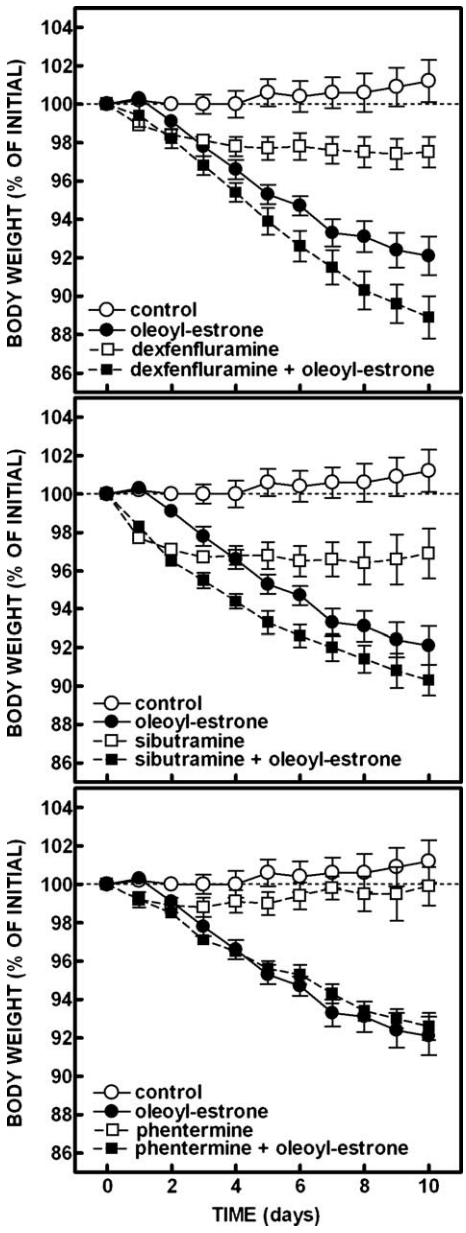

Fig. 1. Body weight change of overweight male Wistar rats receiving a daily oral dose of oleoyl-estrone and/or sibutramine or dexfenfluramine. The data are the mean ± S.E.M. of six different animals and are expressed as percent of the initial body weight. Upper panel: controls and oleoyl-estrone treated rats compared with dexfenfluramine and dexfenfluramine+oleoyl-estrone-treated rats. Center panel: controls and oleoyl-estrone treated rats compared with sibutramine and sibutramine+oleoyl-estrone-treated rats. Lower panel: controls and oleoyl-estrone treated rats compared with phentermine and phentermine+oleoyl estrone-treated rats. Statistical significance of the differences between groups (analysis of the variance); NS=not statistically significant ($P>0.05$):

P values	Effect of oleoyl-estrone	Effect of dexfenfluramine	Effect of sibutramine	Effect of phentermine
Final body weight	<0.001	0.009	0.011	NS

Table 1
Body composition on day 10 and food intake of rats treated with oleoyl-estrone and dexfenfluramine, sibutramine or phentermine

Parameter	Units	Gavage	Controls	Dexfenfluramine	Sibutramine	Phentermine	*P* OE	*P* DX	*P* SB	*P* PH
Body energy density	kJ/g	−OE	11.73 ± 0.49	10.08 ± 0.48	10.69 ± 0.45	11.56 ± 0.45				
		+OE	10.63 ± 0.07	10.30 ± 0.45	10.14 ± 0.45	10.19 ± 0.69	NS	0.042	NS	NS
Body energy content	MJ	−OE	4.35 ± 0.29	3.55 ± 0.20	3.87 ± 0.25	4.46 ± 0.37				
		+OE	3.51 ± 0.08	3.32 ± 0.21	3.21 ± 0.29	3.51 ± 0.18	0.001	0.037	NS	NS
Body lipid	% BW	−OE	17.2 ± 1.1	14.1 ± 1.4	14.2 ± 1.9	18.7 ± 1.6				
		+OE	15.3 ± 0.7	14.5 ± 1.9	13.6 ± 1.1	14.3 ± 1.0	NS	NS	NS	NS
Body lipid pool	g	−OE	63.8 ± 5.0	49.7 ± 5.5	51.5 ± 7.2	71.3 ± 5.6				
		+OE	50.4 ± 1.3	47.0 ± 7.3	43.4 ± 5.2	50.0 ± 4.8	0.009	NS	NS	NS
Body lipid change	% of initial lipid	−OE	0.6 ± 5.5	−20.2 ± 7.8	−20.7 ± 9.7	8.3 ± 9.5				
		+OE	−17.5 ± 3.7	−24.7 ± 10.6	−28.5 ± 5.9	−23.0 ± 5.3	0.010	NS	0.039	NS
Food intake	g/day	−OE	16.6 ± 0.1	13.3 ± 0.7	12.4 ± 0.7	16.9 ± 0.4				
		+OE	11.6 ± 0.6	8.7 ± 0.7	9.5 ± 0.8	12.3 ± 0.4	<0.001	0.003	0.003	NS

The data are the mean ± S.E.M. of six animals per group. BW=body weight; ±OE plus/minus oleoyl-estrone in the gavage. Significance of the differences between groups (analysis of the variance): *P* values for the overall effects of oleoyl-estrone (*P* OE), dexfenfluramine (*P* DX), sibutramine (*P* SB), and phentermine (*P* PH). NS=not statistically significant ($P>0.05$). There were no significant interactions between the different treatments (analysis of the variance).

(Table 2). Insulin decreased by oleoyl-estrone treatment, no other drug affected this parameter. The maintenance of glycaemia and decrease in insulinaemia resulted in a decrease in the HOMA score (i.e., higher insulin sensitivity) in all oleoyl-estrone-treated groups. Oleoyl-estrone treatment, alone or in combination with other drugs, decreased the triacylglycerol and cholesterol levels. Dexfenfluramine also decreased the levels of triacylglycerols and cholesterol, and these effects were additive to those induced by oleoyl-estrone. Dexfenfluramine also slightly increased the 3-hydroxybutyrate levels, a parameter unaffected by the other drugs tested.

No significant effects were observed on aspartate and alanine transaminase levels in serum induced by the treatments, single or combined, of oleoyl-estrone, dexfenfluramine or sibutramine; but phentermine induced an increase in alanine transaminase.

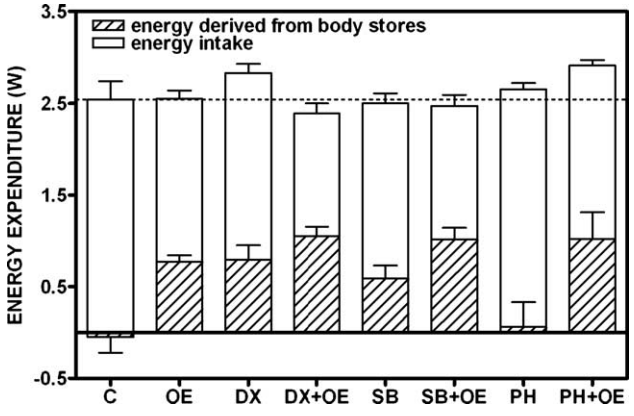

Fig. 2. Energy expenditure of overweight male Wistar rats receiving a daily oral dose of oleoyl-estrone and/or sibutramine or dexfenfluramine. The columns represent the calculated energy expenditure of six animals per group ± S.E.M. The contribution of food intake and internal energy stores is also indicated. The energy intake bar is stacked on top of that representing the energy derived from internal stores. The sum of both bars represents energy expenditure. All units have been converted into W (J/s): (food intake g × 13.3 kJ/g)/day for energy intake, and (energy accrual MJ)/10 days for decrements in body energy. C=controls; OE=oleoyl-estrone; DX=dexfenfluramine; DX+OE=dexfenfluramine+oleoyl-estrone; SB=sibutramine; SB+OE=sibutramine+oleoyl-estrone; PH=phentermine; PH+OE=phentermine+oleoyl-estrone. Statistical significance of the differences between groups (analysis of the variance); NS=not statistically significant ($P>0.05$):

P values	Effect of oleoyl-estrone	Effect of dexfenfluramine	Effect of sibutramine	Effect of phentermine
Energy expenditure	NS	NS	NS	NS
Energy intake	<0.001	0.003	0.003	NS
Energy accrual	<0.001	0.002	0.007	NS

4. Discussion

The rats used in this study contained initially 17% lipid, which corresponds to about 21% of body weight as fat tissue, a condition that can be compared with human overweight (i.e., a body mass index in the range of 30 kg/m^2). The fact that oleoyl-estrone combined with sibutramine resulted in the loss of more than one quarter of its body lipid in just 10 days, without implementing dietary constrains, suggests that the combination of both compounds may facilitate the swift loss of excess fat. Evidently, this is just a first approach to the additive combination of two drugs that have proved separately their efficacy, at least on animal models. The effects of dexfenfluramine and oleoyl-estrone were fully comparable to these, but the caveats that the combination of dexfenfluramine and phentermine carried about must be taken into account (Jollis et al., 2000); however, the lack of alteration of transaminase levels, the maintenance of glycaemia and the concentrations of lipid metabolism indicators in plasma, together with the lowering of insulin resistance (HOMA score) and decreased cholesterol levels suggest that–at least in the short period studied–

Table 2
Plasma composition of rats treated with oleoyl-estrone and dexfenfluramine, sibutramine or phentermine

Parameter	Units	Gavage	Controls	Dexfenfluramine	Sibutramine	Phentermine	*P* OE	*P* DX	*P* SB	*P* PH
Glucose	mM	−OE	8.23 ± 0.13	7.70 ± 0.20	8.21 ± 0.20	8.28 ± 0.26	NS	<0.001	NS	NS
		+OE	8.68 ± 0.17	7.66 ± 0.18	7.95 ± 0.28	8.15 ± 0.17				
Triacylglycerol	mM	−OE	1.79 ± 0.09	1.71 ± 0.08	1.76 ± 0.05	1.83 ± 0.02	<0.001	0.018	NS	NS
		+OE	1.45 ± 0.20	0.91 ± 0.11	1.38 ± 0.15	1.38 ± 0.24				
3-Hydroxybutyrate	μM	−OE	127 ± 6	162 ± 13	110 ± 11	115 ± 10	NS	0.006	NS	NS
		+OE	100 ± 13	130 ± 8	138 ± 22	130 ± 16				
Non-esterified fatty acids	μM	−OE	422 ± 32	410 ± 58	349 ± 19	471 ± 35	NS	NS	NS	NS
		+OE	454 ± 51	391 ± 33	392 ± 31	501 ± 51				
Total cholesterol	mM	−OE	2.26 ± 0.16	2.05 ± 0.05	2.35 ± 0.18	2.12 ± 0.06	<0.001	0.040	NS	NS
		+OE	1.55 ± 0.06	1.35 ± 0.09	1.53 ± 0.09	1.46 ± 0.08				
Asp-transaminase	μkat/L	−OE	2.07 ± 0.21	2.06 ± 0.23	1.77 ± 0.20	1.82 ± 0.24	NS	NS	NS	NS
		+OE	1.88 ± 0.10	1.52 ± 0.33	1.42 ± 0.30	2.05 ± 0.10				
Ala-transaminase	nkat/L	−OE	490 ± 50	467 ± 59	425 ± 27	563 ± 85	NS	NS	NS	0.037
		+OE	374 ± 20	397 ± 22	556 ± 129	590 ± 90				
Insulin	pM	−OE	561 ± 60	574 ± 73	442 ± 65	560 ± 88	0.011	NS	NS	NS
		+OE	384 ± 49	435 ± 45	347 ± 57	494 ± 63				
HOMA score		−OE	28.6 ± 3.1	27.1 ± 2.9	22.8 ± 3.5	28.6 ± 4.4	0.012	NS	NS	NS
		+OE	20.8 ± 2.9	20.8 ± 2.4	16.9 ± 2.8	25.0 ± 3.2				

The data are the mean ± S.E.M. of six animals per group; ±OE plus/minus oleoyl-estrone in the gavage. Significance of the differences between groups (ANOVA): *P* values for the overall effects of oleoyl-estrone (*P* OE), dexfenfluramine (*P* DX), sibutramine (*P* SB), and phentermine (*P* PH). NS=not statistically significant ($P > 0.05$). There were no significant interactions between the different treatments (analysis of the variance).

no significant metabolic alterations were observed, other than the loss of body energy and body weight. This is important, since a large part of the fat susceptible of elimination was already disposed of without the appearance of gross indications of damage or alteration of homoeostatic control.

The combination of oleoyl-estrone with phentermine was much less effective, probably because phentermine alone had only limited effects on body energy: the effects of oleoyl-estrone on body energy were similar in the oleoyl-estrone and phentermine+oleoyl-estrone groups, while the combination of oleoyl-estrone and either dexfenfluramine or sibutramine induced additive effects on food consumption and, consequently, additive effects on body energy losses to maintain energy expenditure unchanged. Most of the energy lost came from lipid, since the changes in other energy components were not statistically significant; this is in agreement with the powerful lipid-mobilising effects, and protein sparing, of oleoyl-estrone (Grasa et al., 2001a) and also agree with the observed mainly lipids loss under sibutramine treatment (van Gaal et al., 1998).

The effects on food intake of oleoyl-estrone and dexfenfluramine or sibutramine were only partially additive, since the combinations of oleoyl-estrone and all other drugs yielded decreases in food intake that were 70–90% of the sum of the effects of the separate treatments. Since the effects of all treatments, alone or combined, did not affect significantly energy expenditure, the effects on food intake became the main factor for lipid mobilisation.

The nil effect of phentermine on food intake contrasts with the marked effects of both serotonin reuptake inhibitors studied (dexfenfluramine and sibutramine) and agree with food intake being controlled essentially by serotonergic pathways (Voigt et al., 2002). The additive effect of oleoyl-estrone indicate that oleoyl-estrone does not act quite on appetite through the modulation of serotonin-controlled pathways, nor does it through modulation of neuropeptide Y (Cabot et al., 1998a) or corticotropin-releasing hormone (Cabot et al., 1998b). Since glycaemia is well maintained, it can be postulated that this maintenance may help decrease food intake, since normoglycaemia under conditions of increased sensitivity to glucose (as is the case in oleoyl-estrone-treated rats) inhibits food intake (Mayer, 1953).

Additional studies should be carried out to explore the safety and medium-/long-term effects on body weight of the combination of oleoyl-estrone and a serotonin reuptake-inhibiting drug such as sibutramine, but the additive results shown here are encouraging. In addition, oleoyl-estrone has been found to prevent or limit the recovery of the fat lost because of its resetting of the ponderostat (Adán et al., 1999), and on the other hand, sibutramine upholds the achieved loss of body weight only when drug treatment is combined with limited energy intake (James et al., 2000). The combination of sibutramine and oleoyl-estrone may help prevent the rapid recovery of the lost fat.

In conclusion, the combination of oleoyl-estrone with a serotonin reuptake inhibitor drug such as sibutramine results in a dramatic loss of body weight and body fat whilst maintaining circulating energy homoeostasis in the over-weight rat.

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References

Adán, C., Cabot, C., Esteve, M., Grasa, M.M., Massanés, R., Vilà, R., Estruch, J., Fernández-López, J.A., Remesar, X., Alemany, M., 1999. Oleoyl-estrone treatment affects the ponderostat setting differently in lean and obese Zucker rats. Int. J. Obesity 22, 366–373.

Alemany, M., Fernández-López, J.A., Pietrobelli, A., Granada, M., Foz, M., Remesar, X., 2003. Pérdida de peso en un paciente con obesidad mórbida en tratamiento con oleoil-estrona (Weight loss in a patient with morbid obesity under treatment with oleoyl-estrone). Med. Clin. 121, 496–499.

Arch, J.R., 1981. The contribution of increased thermogenesis to the effect of anorectic drugs on body composition in mice. Am. J. Clin. Nutr. 34, 2763–2769.

Balada, F., Sanchis, D., Virgili, J., Grasa, M.M., Monserrat, C., Fernández-López, J.A., Remesar, X., Alemany, M., 1997. Effect of the slimming agent oleoyl-estrone in liposomes (Merlin-2) on the body weight of rats fed a cafeteria diet. Arch. Physiol. Biochem. 105, 487–495.

Beynen, A.C., Dekker, D., Gundlach, B.L., Vantintelen, G., 1986. Body-composition of obese rats fed the anorectic drug dexfenfluramine. Nutr. Rep. Int. 33, 491–497.

Blundell, J.E., Hill, AJ., 1992. Dexfenfluramine and appetite in humans. Int. J. Obesity 16, S51–S59.

Bonora, E., Saggiani, F., Targher, G., Zenere, M.B., Alberiche, M., Monauni, T., Bonadonna, R.C., Muggeo, M., 2000. Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity. Studies in subjects with various degrees of glucose tolerance and insulin sensitivity. Diabetes Care 23, 57–63.

Bray, G.A., Greenway, F.L., 1999. Current and potential drugs for treatment of obesity. Endocr. Rev. 20, 805–875.

Cabot, C., Grasa, M.M., Adán, C., Pérez-Clausell, J., Virgili, J., Estruch, J., Fernández-López, J.A., Remesar, X., Alemany, M., 1998a. Oleoyl-estrone does not alter hypothalamic neuropeptide Y. Peptides 19, 1631–1635.

Cabot, C., Grasa, M.M., Estruch, J., Fernández-López, J.A., Remesar, X., Alemany, M., 1998b. Zucker obese rats are insensitive to the CRH-increasing effect of oleoyl-estrone. Brain Res. Bull. 46, 529–534.

Fernández-López, J.A., Remesar, X., Foz, M., Alemany, M., 2002. Pharmacological approaches for the treatment of obesity. Drugs 62, 915–944.

Finer, N., 2002. Sibutramine: its mode of action and efficacy. Int. J. Obesity 26, S29–S33.

Finer, N., Craddock, D., Lavielle, R., Keen, H., 1987. Prolonged weight-loss with dexfenfluramine treatment in obese patients. Diabetes Metab. 13, 598–602.

Folch, J., Lees, M., Sloane-Stanley, G.H., 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 232, 497–509.

Grasa, M.M., Cabot, C., Esteve, M., Yubero, P., Massanés, R.M., Blay, M., Vilà, R., López-Martí, J., Fernández-López, J.A., Remesar, X., Alemany, M., 2001a. Daily oral oleoyl-estrone gavage induces a dose-dependent loss of fat in Wistar rats. Obes. Res. 9, 202–209.

Grasa, M.M., Esteve, M., Massanés, R.M., Yubero, P., Blay, M., López-Martí, J., Cabot, C., Vilà, R., Fernández-López, J.A., Remesar, X., Alemany, M., 2001b. Oral gavage of oleoyl-estrone has a stronger effect on body weight in male obese Zucker rats than in female. Diabetes Obes. Metab. 3, 203–208.

Hansen, D.L., Toubro, S., Stock, M.J., Macdonald, I.A., Astrup, A., 1999. The effect of sibutramine on energy expenditure and appetite during chronic treatment without dietary restriction. Int. J. Obesity 23, 1016–1024.

Heal, D.J., Aspley, S., Prow, M.R., Jackson, H.C., Martin, K.F., Cheetham, S.C., 1998. Sibutramine: a novel anti-obesity drug. A review of the pharmacological evidence to differentiate it from D-amphetamine and D-fenfluramine. Int. J. Obesity 22 (Suppl. 1), S18–S28.

James, W.P.T., Astrup, A., Finer, N., Hilsted, J., Kopelman, P., Rössner, S., Saris, W.H.M., van Gaal, L., 2000. Effect of sibutramine on weight maintenance after weight loss: a randomised trial. Lancet 356, 2119–2125.

Jollis, J.G., Landolfo, C.K., Kisslo, J., Constantine, G.D., Davis, K.D., Ryan, T., 2000. Fenfluramine and phentermine and cardiovascular findings-effect of treatment duration on prevalence of valve abnormalities. Circulation 101, 2071–2077.

Mancini, H.A., 2003. Treatment of obesity: an update on anti-obesity medications. Obes. Rev. 4, 25–42.

Matthews, D.R., Hosker, J.P., Rudenski, A.S., Naylor, B.A., Teacher, D.F., Turner, R.C., 1985. Homeostasis model assessment: insulin resistance and B cell function from fasting plasma glucose and insulin concentration in man. Diabetologia 28, 412–419.

Mayer, J., 1953. Glucostatic mechanism of regulation of food intake. N. Engl. J. Med. 249, 13–16.

Remesar, X., Fernández-López, J.A., Blay, M.T., Savall, P., Salas, A., Díaz-Silva, M., Esteve, M., Grasa, M.M., Alemany, M., 2002. Effect of oral oleoyl-estrone on adipose tissue composition in male rats. Int. J. Obesity 26, 1092–1102.

Rich, S., Shillington, A., McLaughlin, V., 2003. Comparison of survival in patients with pulmonary hypertension associated with fenfluramine to patients with primary pulmonary hypertension. Am. J. Cardiol. 92, 1366–1368.

Ryan, D.H., 2004. Clinical use of sibutramine. Drugs Today 40, 41–54.

Sachdev, M., Miller, W.C., Ryan, T., Jolis, J.G., 2002. Effect of fenfluramine-derivative diet pills on cardiac valves: a meta-analysis of observational studies. Am. Heart J. 144, 1065–1073.

Samanin, R., Garattini, S., 1993. Neurochemical mechanism of action of anorectic drugs. Pharmacol. Toxicol. 73, 63–68.

Sanchis, D., Balada, F., Grasa, M.M., Virgili, J., Peinado, J., Monserrat, C., Fernández-López, J.A., Remesar, X., Alemany, M., 1990. Oleoyl-estrone induces the loss of body fat in rats. Int. J. Obesity 20, 588–594.

Turner, P., 1990. Dexfenfluramine. Its place in weight control. Drugs 39, 53–62.

van Gaal, L.F., Wauters, M.A., Peiffer, F.W., de Leeuw, I.H., 1998. Sibutramine and fat distribution: is there a role for pharmacotherapy in abdominal/visceral fat reduction? Int J. Obesity 22 (suppl. 1), S38–S40.

Voigt, J.P., Schade, R., Fink, H., Hörtnagl, H., 2002. Role of 5-HT1A receptors in the control of food intake in obese Zucker rats of different ages. Pharmacol. Biochem. Behav. 72, 403–409.

Wellman, P.J., Maher, T.J., 1999. Synergistic interactions between fenfluramine and phentermine. Int. J. Obesity 23, 723–732.

Zychlinski, L., Montgomery, M.R., 1984. Alterations in monoamine oxidase activity after single and repeated exposure of rats to chlorphenthermine. Toxicol. Lett. 22, 133–138.