Eur J Nutr 38:247–253 (1999)

ORIGINAL CONTRIBUTION

X. Remesar
V. Tang
E. Ferrer
C. Torregrosa
J. Virgili
R.M. Masanés
J.A. Fernández-López
M. Alemany

Estrone in food: a factor influencing the development of obesity?

Received: 6 April 1999
Accepted: 15 October 1999

X. Remesar · V. Tang · E. Ferrer
C. Torregrosa · J. Virgili · R.M. Masanés
J.A. Fernández-López
Dr. M. Alemany (✉)
Departament de Bioquímica i Biologia Molecular
Facultat de Biologia, Universitat de Barcelona
Av. Diagonal, 645
E-08028 Barcelona, Spain
e-mail: alemany@porthos.bio.ub.es.

Summary *Background* Estrone is a relatively abundant hormone widely distributed in tissues of animal and plant origin. It is a mild estrogen that induces increases in body weigt in experimental animals. The relative abundance of estrone esters in animal tissues suggests that it may also be found in foods, from which it may alter the mechanisms of body weight control.
Aim of the study To measure the total estrone content in food and to determine whether this may affect body weight.
Methods In the first part of the study, a method was devised for the measurement of total estrone content in food. This was applied to the analysis of estrone content in a variety of food. Finally, hyperlipidic diets (18.6 MJ/kg) with a total estrone content 0.89 ± 0.21 μmol/kg (control group) and 1.37 ± 0.13 μmol/kg (laced with estrone fatty esters) were given to rats during 15 days, in order to determine the influence of dietary estrone on the body mass. Zucker lean (Fa/?) rats weighing initially 200–215 g were used. The total estrone (essentially as fatty esters) content of food was investigated by combining a dried methanol extraction with saponification and measurement of the free estrone evolved through radioimmunoassay.
Result The content of estrone was zero in some vegetables, but significant in fruits, meats, and especially fats, both of plant and animal origin. The application of these analyses to a standard recommended diet for humans may result in intakes of more than 1 μmol of estrone per day, a figure comparable to the estrogen production by women. When rats were exposed to a raised estrone content in a fat-rich diet, they significantly increased their body weights, doubling their rate of growth (1.99 g/day) compared with controls (0.81 g/day), but maintaining their plasma composition and the proportions of lipid, water, and protein in their carcasses.
Conclusion The widely distributed estrone esters in food and their relatively high concentrations may result in high free hormone intakes in humans. The continued and massive intake of estrone may enhance tissue deposition and lead to obesity.

Key words Estrone – estrogen – obesity – dietary estrogen

European Journal of Nutrition, Vol. 38, Number 5 (1999)

Introduction

Estrogenic activity, common in the environment, is often due to chemicals which are structurally unrelated to steroidal hormones (4). Estrone and other natural estrogens (9) are found, usually in low concentrations, in a wide variety of sources, including food (10). Their eventual effects are considered negligible (10) since the direct estrogenic activity of estrone is minimal (13). Most of estrone estrogenic effects are a consequence of its *in vivo* conversion to β-estradiol (17).

The literature on the effects of estrone is sparse, despite some interesting traits such as its raised levels in the plasma of the obese (5) and high urinary (11, 12) or fecal (3, 26) excretion rates. Estrone has been found to induce body weight increases in rats (20), and its effects *in vivo* are markedly different from those of typical estrogens such as β-estradiol (14). The finding that fatty acyl-estrone is present in plasma at comparatively high levels for a hormone derivative (7), and the postulated role for oleoyl-estrone as part of the ponderostat system (20), coincide in suggesting that this steroid plays a more important role than currently assumed. This supposition is based on that high estrone levels are widespread in many tissues, including food.

As a result of the finding that estrone is a significant dietary component of Western diet, we decided to test the effect of known amounts of esterified estrone added to the diet of rats, to check whether estrone consumed in this way causes significant changes in body weight, since we already know that the i.v. administration of estrone results in body weight gain in rats (20). This effect is not related to estrogenicity, since estrogen administration tends to decrease – albeit temporarily – body weight (6). The i.v. administration of oleoyl-estrone, however, causes severe losses of weight, mainly fat (21). Again this effect could not be traced to the estrone moiety, since the effect of other esters is considerably lower or zero (19), and free estrone itself induces the reverse effect (20).

In the present paper we have adapted a previously published method for estimating circulating levels of fatty acyl-estrone (2) for the analysis of estrone fatty esters in food and tissue samples. We also checked whether the levels found in human food may influence the deposition of living matter in young rats.

Materials and methods

Measurement of esterified estrone in foods

Food samples were minced, weighed, and mixed with a large amount of anhydrous sodium sulfate (Panreac, Montcada, Spain), used as dehydrating agent, in gross excess of the water content of the samples. The samples were extracted in a 200 ml Soxhlet apparatus (Behr Labor-Technik, Düsseldorf, Germany) with pure anhydrous methanol (Panreac) (20 ml methanol per g of sample) for 6 hours (i.e., about 16 cycles). The methanol extract was filtered through a column of anhydrous sodium sulfate and then stored at –20 ºC until processed. Aliquots of 1 ml were transferred to tubes (in duplicate), and 2 ml trichloromethane (Panreac) was added. After mixing, 1 ml of 3.9 mM $MgCl_2$ was added and the tubes were maintained in a rotary shaker for 2 hours. The phases were separated through centrifugation and the upper phase was discarded. The organic phase was dried in a spedvac vacuum-drying centrifuge (Jouan, Saint Herblain, France). The residues were then resuspended in 1 ml of 0.6 M KOH in ethanol: water (95:5). The tubes were capped and heated for 20 min at 85 ºC. Then 1 ml of water was added and they were shaken and cooled. The samples were extracted twice with 2 ml of peroxide-free ethyl ether (Panreac), and the upper organic phases were combined, neutralized to pH 7 with 100 mM KH_2PO_4, centrifuged, and the new upper phase (ethyl ether) dried in a spedvac vacuum-drying centrifuge. The residue was resuspended in 375 µL ethanol (Panreac). Aliquots of 5–20 µl of this solution were transferred to the final radioimmunoanalysis tubes and mixed with 0.35 ml of 50 mM phosphate buffer pH 8.0. They were used for the measurement of estrone using a standard estrone radioimmunoassay method (Sigma product sheet 9086, 1996) in a medium supplemented with 0.1 % gelatin as support for the estrone microprecipitates, and a polyclonal specific rabbit antiserum raised against estrone-6-thyroglobulin (E-3135 from Sigma, St Louis, MO, USA) (2). In this assay, samples, labeled estrone (DuPont NEN, NJ, USA) – and the standards – were mixed with the antibody in a single step, since here the linearity of the response of the radioimmunoassay was more important than its ability to detect small concentrations. The assay was linear for final sample (0.1 ml) concentrations in the range of 1 to 100 nM. Under the conditions described, the lower practical limit of detection was 0.2 µmol/kg of fresh material. The cross-reactivity with other steroids was insignificant except for dehydroepiandrosterone, but since most of dehydroepiandrosterone-sulphate was left behind in the first ether extraction, this compound cannot actually interfere with the estrone radioimmunoassay.

The effectiveness of the extraction procedure was checked by using ^{3}H-labeled oleoyl-estrone. This compound was prepared from oleoyl-chloride and ^{3}H-estrone (DuPont NEN, NJ, USA) (18). The recovery of label in the dried methanol extracts was 92 ± 3 % (N=4). The effectiveness of the saponification process and radioimmunoanalysis measurement has been previously published (2).

Effect of dietary estrone on rats

Two groups of female Zucker lean (Fa/?) rats (N=5 each) weighing initially 211 ± 8 g (controls) and 204 ± 5 g

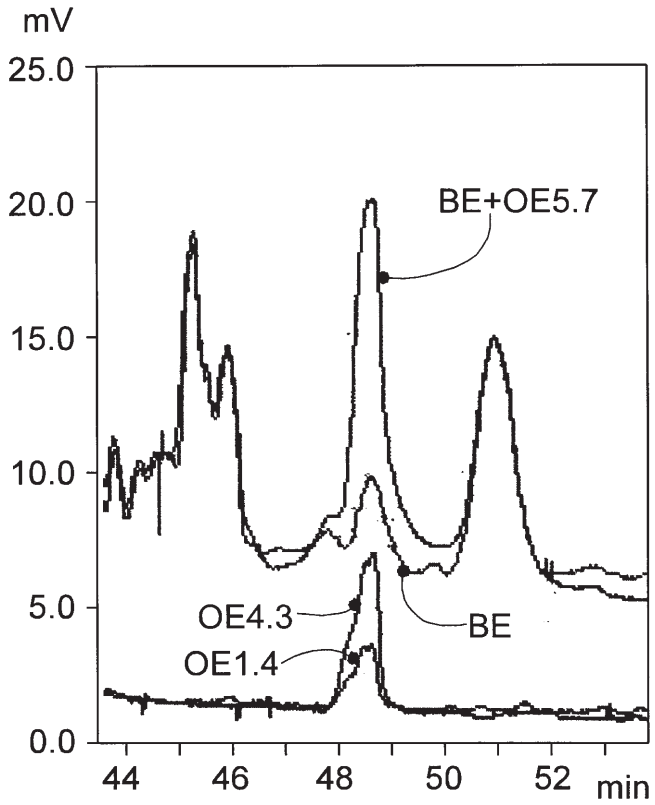

Fig. 1 HPLC chromatogram of a dry methanol extract of butter. The graphs correspond to butter extract (BE), the same extract with an internal standard of 5.7 μg of oleoyl-estrone (BE+OE5.7), and two standards of 1.4 μg (OE1.4) and 4.3 μg (OE4.3) of oleoyl-estrone. An aliquot of butter methanol extracts was chromatographed in an HPLC (Kontron, Milano, Italy) using a Spherisorb ODS 5 mm (250x4.5 mm) (Waters, Milford, MA, USA) column, with a flow of 0.3 ml/min and a pressure of 2.1 kPa. The mobile phase was hexane/isopropanol/methanol (90:10:30).

(treated rats) were kept under standard conditions in metabolic cages (Techniplast Gazzada, Guguggiatte, Italy) and were fed during 15 days a powdered hyperlipidic (18.6 MJ/kg) diet (B&K, Sant Vicent dels Horts, Spain) containing 38 % of its energy as fat, 17 % as protein, and 45 % as starch and other carbohydrates. The diet was prepared to mimic to some extent the high-fat diets consumed by humans. Soy and sunflower oils were the diet's main fat source, in order to minimize the presence of dietary estrone. In any case, its total estrone content (measured) was 0.89 ± 0.21 μmol/kg (N=5). Part of the same diet was supplemented with oleoyl-estrone added to its constitutive oils, the content of this loaded diet being 1.37 ± 0.13 μmol/kg (N=5) of total (acyl-) estrone.

The weights and food consumption of the rats were measured daily. The dose of oleoyl-estrone ingested by the rats was calculated from the food consumed and acyl-estrone content of the diet. At the end of the experiment, the rats were anesthetized with ethyl ether, and blood was taken through heart puncture and used to obtain the plasma. Then the rats were killed and dissected, cleaned of intestinal contents, weighed again, and sealed in polyethylene bags that were subsequently autoclaved at 120 ºC for 2 hours; then, the whole rat was minced to a smooth paste with a blender.

Plasma was used for the estimation of glucose, triacylglycerols, total cholesterol, urea, and total protein using a dry-chemistry strip auto analyzer (Spotchem, Menarini, Milano, Italy), as well as for the estimation of esterified estrone (2). The rat carcass paste was used for the estimation of the proportions of water (differential weighing after 24 h at 100 ºC), lipid (8), energy (bomb calorimeter, C-7000, IKA, Heitersheim, Germany), and protein. The latter was measured as total N with a Carlo Erba NA-1500 elemental analyzer and then converted into protein using a 5.5 factor (16). The total estrone ester content of the carcass paste was also measured using the same procedure used for the diet.

Statistically significant differences ($P < 0.05$) between groups were determined using Student's t test and standard one-way ANOVA programs.

Results

Presence of esterified estrone in food

Aliquots of butter methanol extracts were chromatographed in an HPLC (Fig. 1) and compared with oleoyl-estrone standards (SALVAT,SA, Esplugues de Llobregat, Spain). Since most of the estrone present in plasma is in the form of oleoyl-estrone (unpublished results), we can safely assume that most of the fatty acyl-estrone present in the food samples analyzed was also in this form. This compound becomes, then, a significant component of some fatty foods, at levels which are extremely high for a substance with hormonal effects.

Free estrone levels in foods were found to be extremely low (data not presented), and in most cases undetectable without previous saponification, which means that practically all the estrone we found in food was in the fraction esterified with fatty acids. Very probably, the predominant form was oleoyl-estrone, as observed in our HPLC analysis of butter.

Food total estrone content

Table 1 shows the total estrone content of the 36 foods analyzed, always expressed per kg (or liter in the case of milk) of fresh weight. Only a few foodstuffs – all of them vegetables – showed estrone levels below the practical limit of detection, but the presence of estrone was widespread, both in plant and animal foods. The occurrence of estrone in fats and dairy products was considerable, but

Table 1 Total estrone content of foods. The data are the mean ± SEM of 5 different samples; the radioimmunoassay measurements were carried out in duplicate in different assays. Samples marked § correspond to commercial brands

food types	food	μmol/kg
Chocolates	black chocolate §	0.77 ± 0.25
	hazelnut/chocolate butter §	1.21 ± 0.30
Fish	tuna in vegetable oil (can) §	0.64 ± 0.42
	haddock (dried) §	1.58 ± 0.70
	sardine (fresh)	1.82 ± 0.77
vegetables, fruits and nuts	potato	< 0.25
	lettuce	< 0.25
	tomato	< 0.25
	banana §	0.74 ± 0.40
	apple	0.97 ± 0.36
	almond §	2.00 ± 0.37
pastry	plain cookies §	1.04 ± 0.36
	Danish butter cookies §	1.88 ± 0.70
	scones §	2.66 ± 0.73
eggs	hard-boiled egg	0.86 ± 0.37
	egg yolk	4.85 ± 2.00
meats and meat products	calf's liver	0.58 ± 0.27
	lean pork meat	0.59 ± 0.40
	ham	0.70 ± 0.19
	veal hamburger	0.96 ± 0.29
	veal meat steak	1.03 ± 0.23
	pork sausage	1.86 ± 0.88
	salami §	2.86 ± 1.03
	liver paté §	5.38 ± 2.16
dairy products	fresh cheese (Burgos) §	0.95 ± 0.14
	yogurt §	1.12 ± 0.51
	whole milk §	1.70 ± 0.45
	Swiss cheese (Emmental) §	1.95 ± 0.42
	hard cheese (Parmesan) §	7.81 ± 1.93
oils and fats	sunflower oil §	0.82 ± 0.18
	olive oil §	0.92 ± 0.22
	coconut oil	1.82 ± 0.51
	cocoa butter	2.93 ± 0.55
	lamb fat (fresh)	4.78 ± 1.67
	butter §	9.15 ± 3.25
	pork lard §	11.44 ± 3.04

even fruits contained significant amounts of the hormone. The levels in olive oil may be low compared with butter or lard, but those of cocoa butter or coconut oil are also fairly high, as is the level in almonds and even in fruits (especially when their large water contents are taken into account).

Estimation of human dietary estrone intake

In order to obtain an estimate of the human intake of esterified estrone in food we used as standard diet the dietary guidelines recommended by most texts on dietetics (22). It is very difficult to determine the precise intake of a given compound by human population-groups, since the number of different foods is very high and only a handful of them have been analyzed. Table 2 shows one of these distributions with the size – and energy content – of the portions. We adapted somewhat this dietary breakdown by including several of the foods we analyzed. The results are striking: using this diet, a human being would ingest about 1 μmol of estrone per day.

Effect of dietary estrone on rat body mass and metabolic parameters

The group receiving the hyperlipidic diet (HL) increased its weight steadily for the next two weeks (Fig. 2), with a mean weight gain of 0.81 g/day. The other group received the diet loaded with oleoyl-estrone (HL-OE) and increased its weight faster, with a mean weight gain of 1.99 g/day, the differences being significant ($p < 0.05$) from day 8 onwards. The amount of food eaten by HL rats was 13.1 ± 1.1 g/day, and the amount by HL-OE rats 14.5 ± 1.1 g/day (NS). Thus, the net intake of estrone was about 53 nmol/kg·day in the HL rats and 90 nmol/kg·day in the HL-OE rats. The carcass analysis of the animals (Table 3) showed that there were no significant differences in the percentages of lipid, water or protein between groups, which represents a higher and harmonic

Fig. 2 Increases in body weight of 5 Zucker lean rats receiving the hyperlipidic diet (HL) and 5 receiving the same diet supplemented with oleoyl-estrone (HL-OE). The HL diet contained 0.89 μmol/kg of esterified estrone, and the HL-OE diet contained 1.37 μmol/kg. The differences between both groups (N=5 rats each) were significant ($p < 0.05$) from day 8 onwards.

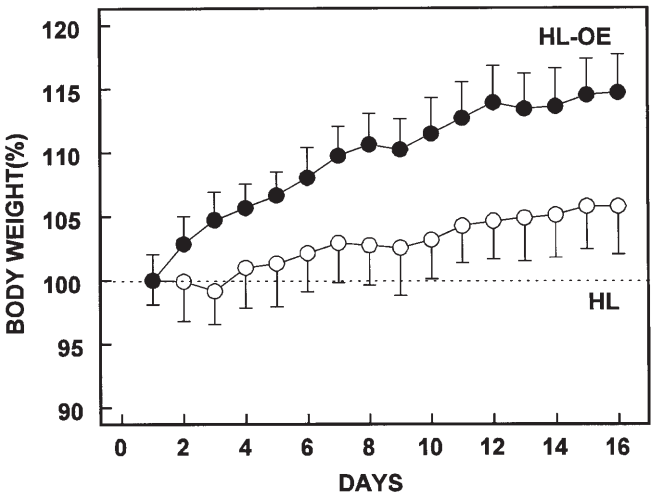

Table 2 Calculated estrone intake in a standard human diet

food group	food and (portions)	g	kcal	nmol E_1	% E_1
cereals *9 portions*	pasta (4)	520	760	*0**	5.6
	bread (2)	50	130	*0**	
	cookies (1)	28	145	29	
	Danish cookies (1)	28	110	53	
vegetables *4 portions*	potatoes (1½)	204	180	0	0
	lettuce (1)	163	20	0	
	carrot (1)	156	70	*0**	
	tomato (½)	62	13	0	
fruits *3 portions*	apple (1)	138	80	134	15.0
	orange (1)	131	60	*0**	
	banana (1)	114	105	84	
dairy products *2½ portions*	milk (1)	244	150	415	46.6
	yogurt (½)	114	70	128	
	hard cheese (½)	14	65	109	
	Swiss cheese (½)	14	55	27	
meats and protein foods *2½ portions*	meat (1)	85	325	88	12.8
	eggs (1)	50	80	43	
	ham (¼)	21	35	15	
	salami (¼)	14	36	40	
oils and fats *¼ portions*	butter (¼)	32	225	293	20.0
totals		2182	2714	1458 §	100.0

* Value not measured.
§ Estimated daily content of estrone in the food chosen. This is a minimal value for the actual content, since the estrone content of several components is unknown, or lower than the limit of detection of the method used.
Dietary guidelines, weight and energy of portions were taken from the "food pyramid" published by the US Dept. of Health and cited in (14); the estimated estrone content was calculated from the data in Table 1.

increase in body mass (i.e., lipid and protein) in the rats receiving the higher dose of dietary acyl-estrone. The complete uniformity of plasma parameters (Table 4) suggests that this enhanced growth in body mass does not affect the circulating levels of triacylglycerols, glucose or urea/proteins.

Discussion

The method described allowed the extraction of fatty acyl-estrone esters from minced food samples, followed by their quantitative saponification and later measurement of the freed estrone nucleus by means of a sensitive radioimmunoanalysis. Losses of material through the whole process were assumed to be low because of the high recoveries of labeled oleoyl-estrone. The sensitivity of the method enabled the measurement to be carried out in small samples of a few milligrams if necessary. The specificity of the method relies on three differential steps: a) solubility in hot methanol, b) saponifiability, and c) specific antigen-antibody interaction. Thus, despite being immersed in a large matrix of other lipids: triacylglycerols, phospholipids, cholesterol, and its esters, none of these compounds complied with the three differential characteristics upon which it is based. In the extractive process all protein, most sugars, and other non-lipophilic compounds were left behind. The saponification process broke down most triacylglycerol and other esters, but fatty acid soaps, hydrophilic materials, and charged molecules remained in the aqueous fraction, while all other compounds, including freed estrone, were removed in the ether phase. The mass of lipid was thus greatly reduced, but after drying and introduction of a buffer solution, lipophilic molecules separated from the aqueous phase and did not interfere further, while estrone was dissolved and interacted with the antibodies. The use of an immunologic method helped minimize interference from other materials found in the samples, a possibility that could not be completely ruled out in spite of repeated checking of the measurements by internal standards in the samples. The results were fairly repeatable even allowing for the elaborate extraction procedure and the use of a radioimmunoassay in the final step of measurement.

Table 3 Body composition of Zucker lean rats fed an hyperlipidic diet supplemented with acyl-estrone for 15 days

parameter	units	HL	HL-OE
initial weight	g	211 ± 8	204 ± 5
final weight	g	224 ± 10	233 ± 6
body weight increase	g / 15 d	13.9 ± 0.3	28.9 ± 1.6*
water content	%	59.2 ± 1.1	59.3 ± 1.5
lipid content	%	12.6 ± 0.9	13.4 ± 1.1
protein content	%	18.9 ± 1.2	20.5 ± 2.4
energy content	kJ / g	11.3 ± 0.7	11.6 ± 0.4

N = 5 animals per group; Statistical significance of the differences between groups: * = $P < 0.05$.

Table 4 Plasma composition of Zucker lean rats fed an hyperlipidic diet supplemented with acyl-estrone for 15 days

parameter	units	HL	HL-OE
glucose	mM	8.33 ± 0.23	8.46 ± 0.29
triacylglycerols	mM	1.08 ± 0.29	1.05 ± 0.07
total cholesterol	mM	1.58 ± 0.13	1.49 ± 0.06
urea	mM	7.35 ± 0.24	7.76 ± 0.31
total protein	g / L	62.0 ± 1.6	61.0 ± 1.2
total (acyl-) estrone	nM	98 ± 9	95 ± 11

N= 5 animals per group; no statistically significant differences were found for the parameters studied.

The levels of total estrone in food were in general very high, especially when compared with the free hormone plasma circulating levels in the 0.2-1.5 nM range (23), and even when compared with total plasma estrone in the 100–300 nM range (7, 18). The most striking finding, apart from its high concentrations, is estrone's widespread distribution in plant and animal foods alike. In any case, the presence of estrone in nuts and fruits should not be necessarily a surprise, given that some plant oils (e.g., palm) have been used as an industrial source of estrone.

The figure obtained in our calculation of dietary human intake (of about 1.5 μmol per day) is within the range of estimated daily estrogen production in women (0.4 to 2.5 μmol/day, calculated from the data in reference 23). We do not know how much of this estrone is finally absorbed and integrated into the metabolism, but in any case, the estrone released by esterases should be large and may influence in some way the processes in which estrone is involved. It must be borne in mind that the diet depicted in Table 2 is a *healthy recommended* diet, high in starch and low in fats and protein. It may be safely expected that people exposed to Western diets may in fact ingest several times more estrone than the amount estimated here. Since a large part of dietary estrone is derived from dairy products, and they constitute – with fruit – the backbone of most low-calorie diets, we can also assume that many of the dietary formulations for body weight control, while being low in fats may yet contain a sizeable amount of natural estrone. The high estrone level of milk may give another dimension to its salutary effect on bone preservation – in addition to its being a good source of minerals –, since estrogen is known to protect bone calcium (25).

In rats, the greater amount of estrone fatty esters in the diet of the HL-OE group caused more rapid weight increase; higher loads of estrone esters reversed the trend, however, leading to actual losses of body weight (unpublished results), in line with the well-known slimming effects of oleoyl-estrone (20, 21). This effect may be probably due to the estrone moiety freed by esterases, since in both groups of rats no changes were observed in the plasma acyl-estrone levels in spite of almost double exposure to the dietary compound.

The mean value of total estrone intake for the human standard diet was about 16 nmol/kg·day (for a 70 kg individual). When the data were corrected by an allometric factor: $BW^{0.75}$ (22), the data were closer to those of the experimental animals: HL 36 nmol/$kg^{0.75}$·day, HL-OE 62 nmol/$kg^{0.75}$·day, and the diet calculated for humans, 46 nmol/$kg^{0.75}$·day. Thus, the estrone intake of humans (corrected by overall metabolic activity) is near or within the range at which it causes significant increases in body weight in rats. The widely distributed estrone esters in food and their relatively high concentrations may result in high hormone intakes in humans.

The data presented add new facts to the known fattening effect of estrone (20), the difference being the range at which this effect occurs and the fact that here the estrone was given as esters included in the diet. The continued and massive intake of estrone may result in an alteration of the ponderostat setting, and so cause fat deposition and lead to obesity in rats (1). In spite of the experimental part of this study having been developed using only experimental animals, the levels of estrone found in common foods, and its widespread distribution, strongly point toward a possible influence of estrone on human fat accretion. The wide consumption of some foods of high estrone content may be a factor contributing to the widespread distribution of obesity. More work is needed to ascertain the possible importance of this newly found dietary hazard, and on the availability and biological effects of dietary estrone fatty esters. These preliminary results strongly emphasize the need to widen the study of the effects of estrone on fat deposition before we can fully attribute obesity-inducing properties to dietary estrone, but the present data point precisely in that direction.

Acknowledgements This work was financed by the Plan Nacional de Ciencia de los Alimentos (ALI96-1094) of the Government of Spain and Laboratoris SALVAT, SA. Thanks are given to Michael Eande from the Language Advisory Service at the University of Barcelona for correction of the text. The study was carried out within the framework of the EC Network on Metabolic Integration and Energy Control ERBCHRX-CT94-0490.

References

1. Adán C, Cabot C,Esteve M, Grasa MM, Masanés R, Vilà R, Estruch J, Fernández-López JA, Remesar X, Alemany M (1999) Oleoyl-estrone treatment lowers the ponderostat setting in lean but not in obese Zucker rats. Int J Obesity (in press)
2. Ardévol A, Virgili J, Sanchis D, Adán C, Fernández-Real JM, Fernández-López JA, Remesar X, Alemany M (1997) A method for the measurement of plasma estrone fatty ester levels. Anal Biochem 249:247–250
3. Adlercreutz H, Gorbach SL, Goldin BR, Woods MN, Dwyer JT, Hämäläinen E (1994) Estrogen metabolism and excretion in oriental and caucasian women. J Nat Cancer Inst 86:1076–1082
4. Bingham SA, Atkinson C, Liggins J, Bluck L, Coward A (1998) Phytoestrogens: where are we now? Br J Nutr 79:393–406
5. Brind J, Strain G, Miller L, Zumoff B, Vogelman J, Orentreich N (1990) Obese men have elevated plasma-levels of estrone sulfate. Int J Obesity 14:483–486
6. Dubuc PU (1985) Effects of estrogen on food intake, body weight, and temperature of male and female obese mice. Proc Soc Exp Biol Med 180:468–473
7. Fernández-Real JM, Sanchis D, Ricart W, Casamitjana R, Fernández-Castañer M, Soler J, Alemany M (1999) Plasma oestrone-fatty acid ester levels are correlated with body fat mass in humans. Clin Endocrinol 50:253–260
8. Folch J, Lees M, Sloane-Stanley GH (1957) A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 232:497–509
9. Harborne JB (1977) Introduction to Ecological Biochemistry. Academic Press, London, pp 83–102
10. Hartmann S, Lacorn M, Steinhart H (1998) Natural occurrence of steroid hormones in food. Food Chem 62:7–20
11. Kesner JS, Knecht EA, Krieg EF, Barnard G, Mikola HJ, Kohen F, Gani MM, Coley J (1994) Validations of time-resolved fluoroimmunoassays for urinary estrone 3-glucuronide and pregnanediol 3-glucuronide. Steroids 59: 205–211
12. Klug TL, Bradlow HL, Sepkovic DW (1994) Monoclonal antibody-based enzyme-immunoassay for simultaneous quantitation of 2-alpha-hydroxyestrone and 16-alpha-hydroxyestrone in urine. Steroids 59:648–655
13. Lipsett M (1986) Steroid hormones. In: SSC Yen, RB Jaffe (eds) Reproductive Endocrinology: Physiology, Pathophysiology and Clinical Management, 2nd edn. WB Saunders, Philadelphia, pp 140–153
14. Lobo MJ, Remesar X, Alemany M (1993) Effect of chronic intravenous injection of steroid hormones on body weight and composition of female rats. Biochem Mol Biol Int 29:349–358
15. Mahan LK, Escott-Stump S (eds) (1996) Krause's Food, Nutrition and Diet Therapy, Saunders, Philadelphia, pp 1040–1115
16. Rafecas I, Esteve M, Fernández-López JA, Remesar X, Alemany M (1994) Whole rat protein content estimation: applicability of the Nx6.25 method. Br J Nutr 72:199–209
17. Ross GT, Vande Wiele RL (1974) The ovaries. In: RH Williams (ed) Textbook of Endocrinology. Saunders, Philadelphia, pp 368–442
18. Sanchis D, Adán C, Ardévol A, Grasa MM, Cabot C, Balada F, Vilà R, Estruch J, Puerta ML, Fernández-López JA, Remesar X, Alemany M (1997) Short-term treatment with oleoyl-estrone in liposomes (Merlin-2) strongly reduces the expression of the ob gene in young rats. Biochem J 326:357–360
19. Sanchis D, Balada F, Farrerons C, Virgili J, Grasa MM, Adán C, Esteve M, Cabot C, Ardévol A, Vilà R, Fernández-López JA, Remesar X, Alemany M (1998) Structural determinants of oleoyl-estrone slimming effects. Life Sci 62:1349–1359
20. Sanchis D, Balada F, Grasa MM, Virgili J, Peinado J, Monserrat C, Fernández-López JA, Remesar X, Alemany M (1996) Oleoyl-estrone induces the loss of body fat in rats. Int J Obesity 20:588–594
21. Sanchis D, Balada F, Picó C, Grasa MM, Virgili J, Farrerons C, Palou A, Fernández-López JA, Remesar X, Alemany M (1997) Rats receiving the slimming agent oleoyl-estrone in liposomes (Merlin-2) decrease food intake but maintain thermogenesis. Arch Physiol Biochem 105:663–672
22. Schmidt-Nielsen K (1975) Animal Physiology. Cambridge University Press, London, pp 208–258
23. Tagatz GE, Guspide E (1973) Hormone secretion by the normal human ovary. In: Handbook of Physiology. The Female Reproductive System. Sect. 7 vol.2 part 1. American Physiological Society, Bethesda, pp 603–613
24. Thomas PR (1996) Guidelines for dietetic planning. In: LK Mahan, S Escott-Stump (eds) S Krause's Food, Nutrition and Diet Therapy. Saunders, Philadelphia, pp 343–370
25. Turner RT, Riggs BL, Spelsberg TC (1994) Skeletal effects of estrogen. Endocr Rev 15:275–300
26. Vos EA (1996) Direct ELISA for estrone measurement in the feces of sows: prospects for rapid, sow-side pregnancy diagnosis. Theriogenology 46:211–231