Eur J Nutr 38:99–104 (1999)

ORIGINAL CONTRIBUTION

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Leptin enhances the synthesis of oleoyl-estrone from estrone in white adipose tissue

Received: 9 November 1998
Accepted: 26 February 1999

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Summary *Background:* Oleoyl-estrone elicits powerful slimming effects on lean and obese rats, sparing protein, lowering appetite and maintaining energy expenditure. Leptin synthesis is markedly reduced by oleoyl-estrone. However, this effect is not observed in the obese Zucker fa/fa rats; these rats do not fully respond to leptin but they lose fat under oleoyl-estrone treatment.
Aim of the study: To determine the role of leptin in the conversion of estrone to fatty-acyl estrone in white adipose tissue both *in vivo* in Zucker lean and obese rats, and *in vitro*.
Methods: Two series of experiments were performed: a) Growth and differentiation of 3T3L1 preadipocytes into adipocytes followed by incubation with tritium-labeled estrone in the medium in the presence / absence of 1 nM leptin, and estimation of the incorporation of label into estrone and estrone ester fractions of cell extracts. b) Zucker lean (Fa/?) [ZL] and obese (fa/fa) [ZO] rats were injected i.v. with carrier-free oleoyl-estrone in chylomicra-sized liposomes, then euthanized after 10 min. Free and esterified estrone were measured in blood, liver, muscle, skin, white adipose tissue (WAT), and brown adipose tissue (BAT).
Results: In the first study, in a 72-h incubation, adipocytes took up 20-27 % of the medium estrone. In the leptin(-) controls, 47 % of the label in the cell fraction was in the form of estrone esters and 45 % as free estrone; in the leptin(+) cells, 71 % of the label was in the estrone ester fraction and 24 % was free estrone. In the second study, a large part of the injected tritium-label remained in the ZO blood, with only a small part remaining in ZL. In ZL 39 % of the label was found in the tissues in the form of free estrone, and in ZO only 22 %; in both cases about half of it was in WAT. Plasma free estrone levels were 0.3±0.1 nM in ZL and 0.5±0.3 nM in ZO, and esterified estrone was 242±99 nM for ZL and 201±29 nM for ZO. Plasma leptin levels were 1.73±0.16 ng/ml in ZL and 61.0±1.4 ng/ml in ZO.
Conclusion: The presence of an intact leptin pathway is critical for the uptake and synthesis of estrone esters as well as for the plasma acyl-estrone turnover. The presented results show a direct relationship between oleoyl-estrone and leptin in the WAT. A fully functional leptin pathway is needed for the synthesis of acyl-estrone and the removal of free estrone from the bloodstream, as well as for the disposal of excess circulating oleoyl-estrone. This has a direct bearing on human and animal obesity, since estrone induces increases in fat deposition.

Key words Obesity – oleoyl-estrone – leptin – Zucker *fa/fa* rat – white adipose tissue

Introduction

Oleoyl-estrone is present in human lipoproteins, its levels being relative to the fat mass (12). The administration of this hormone in liposomes (Merlin-2) exerts powerful slimming effects on lean (24) and dietary obese (25) or genetically obese (6) rats, sparing protein, lowering appetite, and maintaining energy expenditure (24). Oleoyl-estrone has been postulated as a ponderostat signal (24) since it is synthesized by adipose tissue and split by most tissues, which releases free estrone into the bloodstream (23).

Leptin synthesis is markedly depressed by oleoyl-estrone treatment (22). However, this effect is not observed in Zucker fa/fa rats, which maintain high levels of leptin and unchanged expression of the ob gene under oleoyl-estrone treatment (2); in any case fa/fa rats are sensitive to oleoyl-estrone treatment and lose fat when treated (6). Lean rats treated for two weeks with oleoyl-estrone in liposomes continue to maintain the low weight setting achieved in this period for at least one month, while Zucker fa/fa rats begin regaining weight immediately after the treatment ceases (1).

These results point toward a close relationship between the leptin pathway and oleoyl-estrone. The key to understanding the relationship between estrone/oleoyl-estrone and leptin lies in white adipose tissue (WAT) cells. WAT is a key organ in the control of energy management (13), and it is where most of leptin is synthesized (28) and large oleoyl-estrone stores are maintained. In the present study we have determined the influence of leptin on the ability of WAT cells to synthesize and store oleoyl-estrone, a key step in the unravelling of the interrelationship between the putative ponderostat signals leptin and oleoyl-estrone.

Materials and methods

Cell culture studies

Preadipocytes (3T3 L1) from the American Type Culture Collection (Rockville, MA USA) were grown to confluence at 37 ºC in 35 mm multi-well culture dishes in Dulbecco's modified Eagle's medium (DMEM) (Bio-Whittaker Boehringer Ingelheim, Germany) containing 100 ml/l calf serum (Bio-Whittaker) in an incubator under 5 % CO_2. Differentiation was induced 2 days after confluence with 5 mg/L insulin (Sigma St Louis, MO USA), 0.25 µM dexamethasone (Sigma), and 500 µM methyl-isobutyl-xanthine (Sigma) in DMEM containing 100 mL/L fetal bovine serum (Bio-Whittaker). After two days, the xanthine and dexamethasone were removed, maintaining insulin for two additional days. From day 4 after differentiation the cells were incubated in DMEM containing 10 % fetal bovine serum replaced every other day.

The cells were used from 7 to 14 days after differentiation; they were incubated in post-differentiation medium supplemented with 45 nM (111 Mbq/L) of ^{3}H-estrone (Dupont-NEN Boston, MA USA; specific radioactivity 2.5 Tbq/mmol) in the presence or absence of 1 nM recombinant murine leptin (Pepro-Tech, London, UK). The cells were maintained in this medium for 3 days. The cells were then harvested, and washed twice in PBS buffer (40 mM NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4, and 8.1 mM NaH_2PO_4, pH 7.4). An aliquot of the cells suspension was used for the determination of protein (8). Another aliquot was used to determine total radioactivity by scintillation counting. The rest was mixed with 10 volumes of trichloromethane/methanol (2:1 by volume) and extracted overnight under gentle shaking at room temperature in tubes with Teflon-lined caps. The trichloromethane-methanol extract was dried out in a spedvac (Jouan, Saint Herblain, France) vacuum centrifugal evaporator; the dry extract was resuspended in 0.2 ml of dichloromethane.

Cell extracts were injected into an HPLC apparatus (Kontron, Milano, Italy) using a Spherisorb ODS 5 µm (250x4.5 mm) (Waters, Milford, MA USA) column, with a flow of 1 ml/min and a pressure of 13 kPa. The mobile phase was a straight gradient of water: acetonitrile:methanol (50:20:30 at min 0 and 0:20:80 at min 45). Both UV and radioactivity channel measurements were carried out; this way we were able to establish the label present in each fraction (i.e., estrone and estrone esters) referred to cell extracts. No significant radioactivity was found in any of the chromatograms at the elution times corresponding to β-estradiol or estrone sulfate.

In vivo *tissue estrone uptake studies*

Oleoyl-(^{3}H)-estrone was synthesized by us from H^3-estrone (Dupont-NEN) and oleoyl-chloride and incorporated into liposomes as previously described (24).

Female 13-week-old Zucker lean (*Fa/?*) and obese (*fa/fa*) rats from Charles River (France), weighing 233±6 g and 321±9 g, respectively (N=6 per group), were used. The rats were maintained under standard conditions (21 °C, 60–70 % relative humidity, lights on from 08.00 to 20.00), and fed *ad libitum* standard rat chow pellets (B&K, Sant Vicent dels Horts, Spain) and tap water. The rats were handled following the guidelines established by the European Community and the Governments of Catalonia and Spain.

The rats were cannulated, under light ether anesthesia, in the left jugular vein using PE-10 polyethylene tubing (Becton-Dickinson, Parsippanny, NJ USA). They were injected through the cannula with 1 ml/kg of isotonic liposome suspension containing carrier-free oleoyl-estrone labeled with tritium in the steroid moiety (4 pmol/kg of rat weight; i.e., 0.6 MBq/kg).

The rats were killed by decapitation 10 min after the injection, and the blood was recovered in dry heparinized beakers. Samples of liver, blood, periovaric white adipose tissue (WAT), uterus, ovaries, brown adipose tissue (BAT) (interscapular and axilar masses), dorsal skin, and hind leg muscle were dissected and frozen with liquid nitrogen. Aliquots of blood were used to obtain plasma.

Plasma free estrone was measured by direct radioimmunoassay with ^{3}H-estrone (Dupont-NEN) in dried ethyl ether plasma extracts (3), using specific estrone antibodies (E-3135, Sigma). Plasma fatty acyl esters of estrone were measured using this procedure after saponification of lipid extracts (3). Plasma samples were also used for the measurement of plasma leptin with a specific rat leptin radioimmunoassay kit (RL-83K from Linco, St Charles, MO USA).

Tissue samples were kept at -20 ºC until processed. The frozen tissues were minced and extracted with 10–20 volumes of trichloromethane:methanol 2:1 (by volume) for 6 hours in Teflon-sealed screw-cap tubes subjected to gentle rotation. Aliquots of the tissue samples were counted and also used to determine the proportion of intact oleoyl-estrone by separating the free hormone from the ester by thin layer chromatography as described previously (24).

The distribution of label in tissues was corrected for the size of the organs and tissues. Most organs and tissues were weighed, but the mass of blood, skin, muscle, and adipose tissue were calculated or taken from published results relative to similar (sex, age, stock) animals; blood: 5 % of BW (16); muscle: 44 % BW lean (4) and 32 % BW obese (19); total white adipose tissue: 15 % BW lean and 38 % BW obese (6); skin: 17 % BW lean (21) and 12 % BW obese (19). The other organs and tissues studied were weighed. The total amount of free and esterified estrone, and of leptin in the plasma compartment was calculated assuming that blood is 5 % of BW and that plasma constitutes 55 % of blood.

Frozen samples of periovaric white adipose tissue (about 0.5 g) were crushed in a chilled ceramic mortar, weighed, and then introduced into Teflon-sealed capped tubes containing 10 ml of pure methanol and 2 g of anhydrous crystalline Na_2SO_4. The tissue samples were extracted in a rotary rack for 24 h at room temperature. The clear dry methanol extracts were dried in a spedvac vacuum centrifugal evaporator (Jouan); 0.5 ml aliquots of the tissue extract were saponified and then extracted with ethyl ether, the extract being later used for the estrone radioimmunoassay as previously described (3).

Statistical comparisons between means ± SEM were conducted with the Student's *t* test, with a limit of significance set at $p<0.05$.

Results

The radioimmunoassay of the ether extracts allowed the estimation of total esterified estrone in lean rat WAT from different locations: 1.17±0.20 µmol/kg. Previous measurements showed that the content of free estrone was negligible compared with the levels of esterified estrone (unpublished data).

Table 1 shows the quantitative aspects of the HPLC chromatograms of cells incubated with estrone and leptin. The amount of medium estrone that was not washed out in the purification process was about 6.1±0.3 nmol/g protein (measured from cells incubated less than 10 s in the labeled-estrone medium, washed, extracted and used as blank), i.e., about 35 % of the total label in the free estrone peaks of controls. Leptin induced a significant increase in the estrone-ester (i.e., oleoyl-estrone) fraction, with relative decreases in the free estrone peak but with overall increased total radioactivity incorporated into the cells. The results are consistent with leptin inducing the synthesis of fatty-acyl estrone, using as precursor the estrone present in the medium.

Table 2 depicts the distribution of estrone label in lean and obese Zucker rats 10 min after the injection of tritium-labeled oleoyl-estrone. The weight of the organs and tissues studied accounted for 86 % of the body mass in lean rats and 88 % in the obese ones. Maximal total estrone specific activity was found in the liver, BAT, and periovaric WAT in lean rats. In the obese, blood and liver showed the highest specific activities; that of BAT was insignificant, and all other tissues showed lower specific activities than in lean rats. There were no significant differences between lean and obese rats with respect to the esterified versus free estrone ratio in the tissues studied (except in the case of blood). Most of the label was found in the form of oleoyl-estrone, despite the presence of 21.6 % of label as estrone in the lean and 39.4 % in the obese. The amount of esterified estrone label in the blood of obese rats was more than three-fold higher than that in the lean, but the free estrone label was similar in both. The free estrone label present in WAT was about half the total recovered in the tissues studied, being found in higher amounts in the lean than in the obese rats.

The plasma levels (n=6) of leptin were 1.73±0.16 ng/ml (lean) and 61.0±1.4 ng/ml (obese) (P.05); thus, plasma leptin was 35-fold higher in the obese than in lean rats. There were no differences in the levels of plasma total estrone (essentially esterified estrone): 242±99 nM (lean) and 201±29 nM (obese), nor in those of free estrone: 0.3±0.1 nM (lean) and 0.5±0.3 nM (obese). The blood total estrone specific activity observed was 10.2±2.3 Bq/nmol (lean) and 25.0±3.4 Bq/nmol (obese) (P.05); the blood free estrone specific activity was a mean 970 Bq/nmol and that of estrone esters 9.1 Bq/nmol in the lean rats; the obese data were 318 Bq/nmol and 24.2 Bq/nmol, respectively. The amount of label injected was proportional to body weight, but the specific activity of

Table 1 Quantitative distribution of the label in trichloromethane / methanol extracts of adipocytes cells cultured in the presence of labelled estrone without or with leptin added to the medium

fraction	units	leptin (-)	leptin (+)
total estrone radioactivity taken up by cells	% of added label	19.6±1.1	26.6±1.4 *
	pmol/mg protein	29.7±3.1	39.8±2.6 *
estrone peaks	pmol/mg protein	13.3±1.0	9.6±0.9 *
estrone fatty esters peak	pmol/mg protein	14.2±2.0	28.6±3.9 *

The data are the mean±SEM of 6 different wells. The conditions of the experiment are given in the text. Statistical significance of the differences between groups (Student's *t* test): leptin (+) versus leptin (-): * = $p < 0.05$

Table 2 Tissue label distribution of estrone and fatty-acyl esterified estrone in lean and obese Zucker rats 10 min after the injection of ^{3}H-oleoyl-estrone

tissue or organ	tissue or organ mass (g)		tissue specific radioactivity ✧		esterified versus free estrone ratio		label in free estrone fraction (%) ♦		label in esterified estrone fraction (%) ♦	
	lean	obese	lean	obese	lean	obese	lean	obese	lean	obese
blood	11.1±0.3	16.0±0.5 *	1401±406	2762±221 *	7.6±2.1	30.6±10.9 *	1.8±0.5	1.4±0.3	13.7±3.8	42.7±9.3*
liver	7.7±0.2	11.5±0.6 *	3788±478	1372±320 *	2.7±0.9	3.0±2.0	7.9±1.8	4.0±1.8	21.4±4.9	11.8±4.8
WAT	33.2±0.8	111.6±3.0 *	970 §	190 §	0.6±0.2	0.7±0.0	20.1±5.4	12.5±3.1	12.1±3.0	8.7±2.4
BAT	1.7±0.2	0.6±0.4 *	2345±848	49±8 *	0.6±0.1	0.8±0.1	2.4±0.6	0.02±0.00*	1.5±0.4	0.01±0.00*
skin	37.6±1.0	38.3±1.1	615±23	176±29 *	0.4±0.1	0.9±0.4	3.0±0.4	2.5±0.8	1.2±0.2	2.3±0.8
muscle	97.2±2.5	102.0±2.9	90±25	44±10	1.1±0.3	2.6±1.0	4.2±1.2	1.3±0.4	4.6±1.1	3.2±1.0
total body weight	233±6	321±9 *	—	—	—	—	39.4±4.7	21.6±3.3*	54.4±6.5	68.8±10.7

The values are the mean ±SEM of 6 rats. Statistical significance of the differences between groups (Student's *t* test): lean versus obese: * = $p<.05$

✧ Label values are given in $1/10^{5}$ of the labeled estrone dose injected (as oleoyl-estrone: 135 kBq in lean and 170 kBq in the obese rats) per gram of tissue

♦ Percentage of injected label present in the whole tissue

§ Tissue radioactivity for periovaric WAT was 1909±744 (lean) and 203±42 (obese), and that for subcutaneous WAT was 615±180 (lean) and 176±44 (obese). For global calculations the mean values of these two sets of figures were used

estrone label in the blood of obese rats was higher than in the blood of the lean. The specific activity of free estrone showed the reverse, being three fold-higher (although that of estrone esters was only half) in lean than in the obese.

Discussion

Fatty esters of estrone are present in WAT in large amounts compared with other steroid hormone esters, e.g., testosterone (10). It has been previously pointed out that aromatic-ring steroids could not be esterified by the standard pathway in which cholesterol and other steroids yield their fatty acyl-derivatives (17). In fact, most estrogen esters described in tissue extracts, such as 17β-estradiol fatty esters, are not esterified in the aromatic A-ring and show a fair variety of substituting fatty acids, with a predominance of polyunsaturated fatty acids (20).

Zucker obese rats contain a large amount of WAT compared with the lean rats (6); the proportion of free estrone label retained in this large mass, 12.5 % of that injected, was about half of all the label recovered as free estrone, a figure comparable in this aspect with the 20.1 % in the WAT of lean rats.

Zucker fa/fa rats, lacking fully functional leptin receptors, show very high circulating leptin levels (15, 22), and similar circulating oleoyl-estrone levels to the lean (22). This uniformity in acyl-estrone levels contrasts with the direct ratio of circulating fatty-acyl estrone levels to body fat mass found in humans (12); this lower presence of oleoyl-estrone in the blood points toward lower synthesis

of this compound under basal conditions. Thus, the estrone taken up by WAT was in the same range in lean and obese rats. This means that the obese rat cells were less efficient in removing estrone from the bloodstream. This result obtained *in vivo* is consistent with the findings of the study *in vitro*, since the presence of added leptin to the medium resulted in a higher uptake of estrone and enhanced synthesis of acyl-estrone. The presence of an intact leptin pathway was, thus, critical for the uptake and synthesis of estrone esters, as it was for the plasma acyl-estrone turnover.

The disposal of injected oleoyl-estrone tracer is less efficient in obese than in lean rats since its degradation is slower (5, 23); thus, most of it remains intact in the blood compartment. The higher specific activity of blood estrone 10 min after the injection of label in the obese rats suggests that the turnover is faster in the lean – they maintain much less estrone ester in blood – than in the obese. The total amount of label recovered in the form of free estrone was also larger in the lean than in the obese, suggesting that obese rats show a hampered ability to degrade circulating acyl-estrone.

The injection of label did not significantly increase the size of estrone pool, thus the results could not be traced to difficulties in disposal of pharmacological amounts of estrone esters. Nevertheless, the form in which this label was injected – chylomicron-sized lipid droplets – may influence its disposal, since particles of this size are easily filtered out by liver, spleen, and lungs (5, 23). In any case, if we take into account only the label not present in the blood as acyl-estrone, the amount of label retained by the liver with respect to the blood was similar in both groups.

Oleoyl-estrone treatment lowers the expression of the *OB* gene in lean Zucker rats (22), but does not affect the obese (2). This points towards oleoyl-estrone modulating leptin synthesis and activity, and also indicates that oleoyl-estrone slimming properties are not mediated by leptin, since oleoyl-estrone induces weight loss in Zucker *Fa/?* and *fa/fa* rats alike (6). The effect of oleoyl-estrone on leptin synthesis, however, needs fully functional leptin receptors, which implies that the oleoyl-estrone effects on leptin synthesis are mediated somehow by leptin. Notwithstanding, leptin also affects the post-treatment effects of oleoyl-estrone (1) and, as we have shown here, has a marked effect on the recycling of estrone, by affecting the rate of hydrolysis of circulating acyl-estrone and the uptake of estrone by WAT, and the synthesis of acyl-estrone by this tissue. This may explain the low circulating levels of acyl-estrone related to fat mass in the Zucker obese rats under basal conditions, as well as their relative accumulation of free estrone in the plasma compartment. It may help explain the high circulating levels of free estrone and estrone sulfate found in obese humans (9), since obesity is related to decreased leptin sensitivity (17), the "leptin resistance" that limits leptin effects on body weight (27).

There is widespread evidence of leptin acting on WAT metabolic processes (14). The influence of leptin on estrone retrieval from the bloodstream to enhance the synthesis of oleoyl-estrone gives further support to its regulatory role on WAT. Leptin effects are transduced through a specific leptin receptor (18), a process which is defective in Zucker *fa/fa* rats (11). Since leptin levels in these animals are very high, we can assume that the diminished turnover of estrone / acyl-estrone is a consequence of defective reception or transduction of leptin signals – not due to low leptin availability –, which in turn implies that the effects of leptin on acyl-estrone metabolism are mediated by leptin receptors. This effect gives evidence for the presence of leptin receptors in WAT and the closing of a feedback regulatory loop with oleoyl-estrone, both WAT products inducing generalized lipolysis and thermogenesis (14, 25, 26), which result in the loss of fat mass. The well-known effects of leptin on the development of reproductive capacity (7), which is linked to synthesis of estrogen may in some way affect the oleoyl-estrone pathway, providing a further, albeit unexplored, link between estrone/oleoyl-estrone as growth control factors and leptin, a key inducer of development.

The results presented show a direct relationship between oleoyl-estrone and leptin in the WAT. A fully functional leptin pathway is needed for the synthesis of acyl-estrone and the removal of free estrone from the bloodstream, as well as for the disposal of excess circulating oleoyl-estrone. Since estrone induces fat deposition, an impairment in the leptin pathway may result in increased estrone levels and thus induce obesity. The resistance to leptin shown by obese humans, coupled with their high circulating free estrone levels may be directly related to their massive storage of fat.

Acknowledgments This work was financed by Laboratoris SALVAT, SA. and the Plan Nacional de Ciencia de los Alimentos (ALI96-1094) of the Government of Spain. Thanks are given to Robin Rycroft from the Language Advisory Service at the University of Barcelona for correction of the text. The work 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 differently affects the ponderostat setting in lean and in obese Zucker rats. Int J Obesity (in press)
2. Adán C, Grasa MM, Cabot C, Esteve M, Vilà R, Masanés R, Estruch J, Fernández-López JA, Remesar X, Alemany M (1998). Short-term treatment with oleoyl-estrone in liposomes (Merlin-2) does not affect the expression of the ob gene in Zucker obese rats. Mol Cell Biochem (in press)
3. 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
4. Arola L, Herrera E, Alemany M (1979) A method for the estimation of striated muscle mass in small laboratory animals. Rev Esp Fisiol 35:215–218
5. Balada F, Sanchis D, Grasa MM, Virgili J, Estruch J, Fernández-López JA, Remesar X, Alemany M (1998) Differential short-term distribution of estrone and oleoyl-estrone administered in liposomes to lean and obese Zucker rats. Obesity Res 6:34–39
6. Balada F, Sanchis D, Grasa MM, Virgili J, Estruch J, Fernández-López JA, Remesar X, Alemany M (1997) Effect of the slimming agent oleoyl-estrone in liposomes on the body weight of Zucker obese rats. Int J Obesity 21:789–895
7. Barash IA, Cheung CC, Weigle DS, Ren HP, Kabigting EB, Kuijper JL, Clifton DK, Steiner RA (1996) Leptin is a metabolic signal to the reproductive system. Endocrinology 137:3144–3147
8. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantitites of protein utilizing the principle of protein-dye biding. Anal Biochem 72:248–254
9. 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
10. Borg W, Shackleton CHL, Pahuja SL, Hochberg RB (1995) Long-lived testosterone esters in the rat. Proc Nat Acad Sci USA 92:1545–1549
11. Chua SC, Chung WK, Wu-Peng XS, Zhang Y, Liu SM, Tartaglia L, Leibel RL (1996) Phenotypes of mouse diabetes and rat fatty due to mutations in the OB (leptin) receptor. Science 271:994–996
12. Fernández-Real JM, Sanchis D, Ricart W, Casamitjana R, Balada F, Remesar X, Alemany M (1999) Plasma oestrone-fatty acid ester levels are correlated with body fat mass in humans. Clin Endocrinol (in press)
13. Flier JS (1995) The adipocyte: storage depot or node on the energy information superhighway?. Cell 80:15–18
14. Frühbeck G, Aguado M, Martínez JA (1997) In vitro lipolytic effect of leptin on mouse adipocytes: evidence for a possible autocrine/paracrine role of leptin. Biochem Biophys Res Commun 240:590–594
15. Hardie LJ, Rayner DV, Holmes S, Trayhurn P (1996) Circulating leptin levels are modulated by fasting, cold, exposure and insulin administration in lean but not Zucker (fa/fa) rats as measured by ELISA. Biochem Biophys Res Commun 223:660–665
16. Hobbs JT (1967) Total blood volume – its measurement and significance. The Radiochemical Centre, Amersham, pp 1–22
17. Jones DL, James VHT (1985) The identification, quantification and possible origin of non-polar conjugates in human plasma. J Steroid Biochem 22:243–247
18. Lee GH, Proenca R, Montez JM, Carroll KM, Darvishzadeh JG, Lee JI, Friedman JM (1996) Abnormal splicing of the leptin receptor in diabetic mice. Nature 379:632–635
19. Masanés R, Fernández-López JA, Alemany M, Remesar X, Rafecas I (1999) Effect of dietary protein on tissue protein synthesis rates in Zucker lean rats. Nutr Res (in press)
20. Mellon-Nussbaum SH, Ponticorvo L, Schatz F, Hochberg RS (1982) Estradiol fatty acid esters. J Biol Chem 257:5678–5684
21. Remesar X, Arola L, Palou A, Alemany M (1981) Body and organ size and composition during the breeding cycle of rats (*Rattus norvegicus*). Lab Anim Sci 31:67–70
22. 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
23. Sanchis D, Balada F, Grasa MM, Virgili J, Monserrat C, Fernández-López JA, Remesar X, Alemany M (1997) Short-term handling of the slimming agent oleoyl-estrone in liposomes (Merlin-2) by the rat. Mol Cell Biochem 177:153–157
24. 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
25. 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
26. Tuominen JA, Ebeling P, Heiman ML, Stephens T, Koivisto VA (1997) Leptin and thermogenesis in humans. Acta Physiol Scand 160:83–87
27. Zakrzewska KE, Cusin I, Sainsbury A, Rohner-Jeanrenaud F, Jeanrenaud B (1997) Glucocrticoids as counterregulatory hormones of leptin – toward an understanding of leptin resistance. Diabetes 46:717–719
28. Zhang Y, Proenca R, Maffel M, Barone M, Leopold L, Friedman JM (1994) Positional cloning of the mouse obese gene and its human analogue. Nature 372:425–432