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PLASMA ACYL-ESTRONE LEVELS ARE ALTERED IN OBESE WOMEN

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ABSTRACT

A group of obese women (BMI>27 kg/m^2; N=73) was studied together with lean controls (BMI <27 kg/m^2; N=25). Three groups were defined by the compliance with: BMI lower than 27 kg/m^2 , glycaemia lower than 5.5 mM and insulinaemia lower than 0.2 nM (controls, group 1, N=19). The subjects with BMI>27 kg/m^2, glucose >5.5 mM and insulin >0.2 nM constituted group 3 (N=41), and those with BMI>27 with glycaemia and/or insulinaemia lower than the limits set constituted group 2 (N=32). The women in group 3 had higher fat content, BMI and fat-free mass than those in group 2 and the controls. There were no changes in most plasma parameters, such as free estrone and β-estradiol. Leptin levels were higher in groups 2 and 3 than in controls. In controls, leptin and acyl-estrone levels were well correlated with BMI and fat content; this correlation was not found in groups 2 and 3 for acyl-estrone, although it was found for leptin. Acyl-estrone levels were lower than expected in most obese women when compared to those of controls, suggesting an altered availability or function of this hormone. In obese women, acyl-estrone levels –and probably function– are lower than expected, contrasting with maintained leptin-BMI correlations. The role of insulin in the control of body weight, perhaps through acyl estrone-mediated effects, should be re-evaluated.

INTRODUCTION

The regulation of fat deposition by insulin is a key factor in the control of body weight (1). Obesity is characterized by an altered insulin function, mainly through increases in insulin resistance (2), typified by higher secretion, levels and extrahepatic removal (3). The obese also tend to show increased glucose levels, a consequence of insulin resistance, which often develops into diabetes. The altered insulin function is either a consequence of obesity or a causal factor in its development (1); but in any case the intensity of insulin alterations is a distinctive trait of the metabolic consequences of obesity (4,5) and its associated pathology: the metabolic syndrome (6).

Leptin, the product of Ob gene (7) is produced mainly in adipose tissue (7,8), in such a way that the circulating levels can be correlated with the fat mass (9,10). This relationship is somewhat altered in severe obesity in humans (11). In some animal models, such as the ob/ob mice, obesity is the consequence of lack of leptin secretion (7,12) or defects in its receptors –Zucker fa/fa rat, db/db mouse– accompanied by very high circulating leptin levels (13). There is no doubt that leptin is a main factor in the body weight control system (14), as well as an index of fatness (15). Our previous research has shown that oleoyl-estrone (OE), a hormone synthesized in the adipose tissue (16), induces the loss of body fat both in normal (17), dietary-obese (18) and genetic-obese (19) rats, sparing protein and maintaining thermogenesis (20). OE decreases the expression of leptin (21), but leptin enhances the synthesis and storage of OE in adipose tissue (16). In addition, OE administration may alter the ponderostat setting (22), which suggests that OE is a signal from adipose tissue participating downstream of leptin in the system controlling the body weight (22). The circulating levels of OE in normal-weight and mildly obese humans are strongly correlated with the BMI, fat mass and circulating leptin (23), but obese rats show lower OE than expected and altered leptin levels, in line with its purported role as a ponderostat signal (22).

Here we used the degree of alteration of insulin functionality as a parameter for the classification of severe obesity. We used this factor as a correlate of the intensity of energy homeostasis impairment and the alteration of the body weight control system. We attempt to determine whether in severe human obesity the BMI-OE correlation is maintained, or altered as found in obese rats, as a sign of the anomalies affecting the body weight control system.

MATERIALS AND METHODS

Subjects and Study Design

Obese and overweight women (body mass index [BMI]>27 kg/m^2) were recruited among those attending the Clinics of Obesity and Nutrition at the Hospital de St. Joan de Reus (N=73). The control group consisted of normal- and low-weight women (BMI <27 kg/m^2) (N=25), and was recruited from the same patients' pool (women suffering anorexia nervosa but otherwise healthy, N=8) and the medical staff. All subjects were free of inflammatory or infectious disease at the time of the study, had normal thyroid hormone levels and were not receiving anti-inflammatory medication, insulin, or other hormonal treatment. Patients and controls were requested to maintain their normal eating habits in the week prior to the study and all reported that their body weight remained stable over the previous three months.

Fasting blood samples were taken at 8.00 am for biochemical and hormone analyses. Body composition measurements were then performed.

TABLE 1
Essential Measures of the Subjects Included in the Study

parameter	units	group 1 BMI<27; [glc]<5.5 & [ins]<0.2	group 2 BMI>27; [glc]<5.5 or [ins]<0.2	group 3 BMI>27; [glc]>5.5 & [ins]>0.2
N		19	32	41
age	y	29 ± 3	43 ± 2 ‡	44 ± 2 ‡
weight	kg	49.9 ± 2.2	94.0 ± 3.2 ‡	104.7 ± 2.9 ‡*
fat-free mass	kg	38.6 ± 1.3	50.3 ± 1.3 ‡	53.8 ± 1.2 ‡*
fat mass	kg	11.3 ± 1.1	43.8 ± 2.0 ‡	50.0 ± 1.8 ‡*
body fat	% BW	21.8 ± 1.4	46.0 ± 0.7 ‡	47.8 ± 0.5 ‡*
BMI	kg/m^2	19.3 ± 0.8	38.0 ± 1.3 ‡	41.3 ± 1.0 ‡*
waist/hip ratio	-	0.81 ± 0.01	0.89 ± 0.01 ‡	0.90 ± 0.02 ‡

Statistical significance ($p<0.05$) of the differences between groups: ‡ differences between group 1 and groups 2 or 3; * differences between groups 2 and 3

The patients were classified using as discriminating factor both a standard measure of obesity: BMI higher or lower than 27 (24), as well as the glycaemia-insulinaemia data. Three groups were defined. Group 1: BMI lower/equal than 27, glycaemia lower/equal than 5.5 mM and insulinaemia lower/equal than 0.2 mM; i.e. non-obese with normal glycaemia and insulinaemia (N=19); the controls that had a BMI<27 but did not meet both the glycaemia and insulinaemia criteria (N=6) were discarded. Group 2: BMI higher than 27, and either glycaemia lower/equal than 5.5 mM or insulinaemia lower/equal than 0.2 mM; i.e. obese with normal or mildly altered glycaemia and insulinaemia (N=32). Group 3: BMI higher than 27, glycaemia higher than 5.5 mM and insulinaemia higher than 0.2 mM; i.e. obese with altered glycaemia and insulinaemia (N=41) (Table 1).

Anorexic patients were included in the study in order to obtain a wider range of BMI values. No significant differences with the other women in the control group were found for the hormonal parameters studied other than those related to their varying body weight.

The study protocol was authorized by the Ethics Committee of the Hospital St. Joan and each subject included in the study gave a voluntary, fully-informed, written consent.

Body Composition Analyses

Height was measured to the nearest mm with a wall-mounted stadiometer, weight was determined on a standard clinical balance with an accuracy of ± 100 g, and BMI (in kg/m^2) was calculated. Waist girth was measured at the minimum circumference between the iliac crest and the

rib cage, hip girth at the maximum width over the greater trochanters and the waist-to-hip ratio was then calculated.

Whole body impedance at 50KHz was measured using a tetrapolar bioelectrical impedanciometer (Human-Im ScanR, Dietosystem, Spain) as we have described elsewhere (25). All measurements were performed early in the morning, under fasting conditions and after voiding, in accordance with the National Institutes of Health Technology Assessment Conference Statement (26). From these data, fat-free-mass (FFM) was calculated using the gender-specific equations validated by Segal *et al.* (27). Fat mass was the difference between body weight and FFM.

Metabolite and Hormone Analyses

Plasma hormones were measured through radioimmunoassay: free and acyl-esterified estrone (28), leptin (HL81K kit, Linco Research, St Charles, MO USA), insulin (Biotrak RPA547 kit, Amersham, Little Chalfont, UK), and β-estradiol (TKE22 Coat-a-count kit, DPC, Los Angeles CA, USA). Plasma was also used for the measurement of total cholesterol, total protein, glucose, urea, creatinine, uric acid, triacylglycerols and aspartate transaminase activity, by standard automated procedures (ITC Diagnostics IZASA, Sant Andreu de la Barca, Spain); 3-hydroxybutyric acid (310UV kit, Sigma, St Louis, MO USA), free (nonsterified) fatty acids (NEFAC kit, Wako Chem., Neuss, Germany) and lactate (29) were also measured.

Differences in mean (± SEM) values between groups were assessed by unpaired t-test. Correlation and regression analyses were performed for quantitative variables using the SPSS 8.0 program. Statistical significance was accepted at $p<0.05$.

RESULTS

Table 1 includes the anthropometric measurements, BMI and other body composition-derived measures. As expected, there were differences between groups 1 and 2 / 3 for all parameters, but groups 2 and 3 also showed significant differences in weight, fat-free mass, fat tissue mass, percentage of body fat and BMI, but not in waist-hip ratio.

Table 2 shows the plasma levels of metabolites. There were no differences in protein, urea, creatinine, triacylglycerols, free fatty acids or 3-hydroxybutyrate. There were differences between nonobese (group 1) and obese (groups 2 and 3) for glucose, uric acid, and aspartate transaminase activity, all highest in group 3. Cholesterol levels in group 3 were also higher than in group 1. There were also differences between groups 2 and 3 in glucose, lactate and uric acid: in all cases the highest values were those of group 3.

Table 3 presents the levels of circulating hormones in normal-weight and obese women. There were no differences in the levels of free estrone or β-estradiol. Obese (groups 2 and 3) women showed higher levels of insulin and leptin than the nonobese (group 1). Esterified estrone levels in group 3

TABLE 2
Plasma Parameters of Normal-Weight and Obese Women

parameter	units	group 1 BMI<27; [glc]<5.5 & [ins]<0.2	group 2 BMI>27; [glc]<5.5 or [ins]<0.2	group 3 BMI<27; [glc]<5.5 & [ins]<0.2
N		19	32	41
protein (total)	g/L	74 ± 1	73 ± 1	72 ± 1
glucose	mM	4.7 ± 0.1	5.6 ± 0.2 ‡	7.0 ± 0.4 ‡✳
lactate	mM	1.39 ± 0.13	1.32 ± 0.10	1.69 ± 0.08 ✳
urea	mM	5.3 ± 0.4	5.3 ± 0.2	5.7 ± 0.2
uric acid	µM	173 ± 18	220 ± 11 ‡	263 ± 13 ‡✳
creatinine	µM	81 ± 4	74 ± 2	76 ± 1
triacylglycerols	mM	1.5 ± 0.8	1.3 ± 0.1	1.6 ± 0.1
free fatty acids	mM	0.22 ± 0.06	0.28 ± 0.04	0.38 ± 0.05
3-hydroxybutyrate	mM	0.51 ± 0.16	0.47 ± 0.11	0.62 ± 0.19
cholesterol (total)	mM	4.9 ± 0.3	5.4 ± 0.2	5.8 ± 0.2 ‡
aspartate transaminase	IU	0.26 ± 0.02	0.46 ± 0.05 ‡	0.51 ± 0.04 ‡

Statistical significance ($p<0.05$) of the differences between groups: ‡ differences between group 1 and groups 2 or 3; ✳ differences between groups 2 and 3.

TABLE 3
Plasma Hormones in Normal-weight and Obese Women

parameter	units	group 1 BMI<27; [glc]<5.5 & [ins]<0.2	group 2 BMI>27; [glc]<5.5 or [ins]<0.2	group 3 BMI>27; [glc]>5.5 & [ins]>0.2
N		19	32	41
insulin	nM	0.094 ± 0.007	0.26 ± 0.03 ‡	0.63 ± 0.10 ‡✳
glucose/insulin ratio	–	53.2 ± 3.2	16.8 ± 1.7 ‡	30.2 ± 3.9 ‡✳
leptin	µg/L	7.3 ± 1.0	49.6 ± 7.2 ‡	48.3 ± 5.0 ‡
estrone (free)	nM	0.84 ± 0.19	0.83 ± 0.15	0.95 ± 0.12
estrone (esterified)	nM	166 ±15	196 ± 25	215 ±18 ‡
β-estradiol	nM	0.28 ± 0.07	0.27 ± 0.06	0.23 ± 0.03

Statistical significance ($p<0.05$) of the differences between groups: ‡ differences between group 1 and groups 2 or 3; ✳ differences between groups 2 and 3

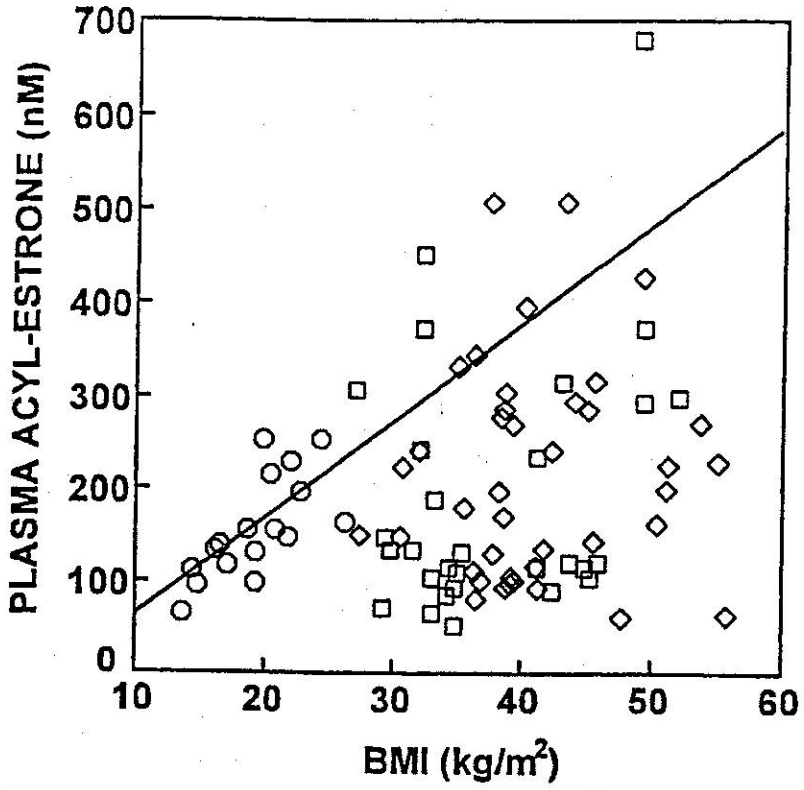

FIGURE 1
Relationship Between Plasma Acyl-estrone Levels and the BMI of Women.

Group 1: circles; Group 2: squares; Group 3: diamonds. The calculated regression line for Group 1 has been drawn. Statistical analysis of the relationships between acyl estrone levels and BMI:

group:	1	2	3
correlation coefficient, r	0.684	0.312	0.045
significance, p	0.003	0.099	0.784

were also higher than in group 1. The only differences between groups 2 and 3 were in insulin and in the ratio of glucose versus insulin, both higher in group 3 but still lower than in group 1.

Figure 1 shows the relationships between plasma acyl-esterified estrone levels and the BMI. The correlation was good for non-obese women (group 1) but this effect was not observed in the obese. Thus there is a clear correlation between acyl-estrone levels and BMI at low BMI values, i.e. this is true for nonobese women, but the linearity disappears with obesity, the values being lower, for most of the women in groups 2 and 3 than expected from the regression line drawn using the nonobese controls.

Figure 2 shows the crossed correlations between some parameters that define obesity and the levels of the main hormones controlling the energy distribution: leptin, insulin and acyl-estrone. Group 1 shows the highest degree of correlation between the data. There is a clear correlation between acyl-estrone and both BMI and the percentage of fat. Leptin levels were also correlated with BMI and

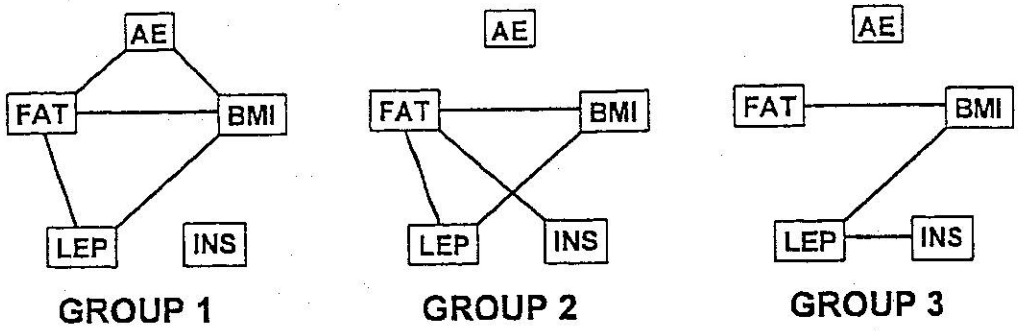

FIGURE 2
Interrelationship Between Body Weight Measures and Hormonal Factors in Lean And Obese Women.

The lines represent a significant relationship between the parameters connected ($p<0.05$); lack of connecting line indicates that no such correlation has been found.
AE = plasma acyl-estrone; FAT = % fats in body weight; BMI = body mass index; LEP = plasma leptin; INS = plasma insulin.

percentage of fat. Insulin was not correlated with either of the other parameters. Leptin maintained its correlation with BMI in groups 2 and 3, but its correlation with percentage of fat was maintained only in group 2. Plasma acyl-estrone was not correlatied with any other parameter in groups 2 and 3. In group 3 insulin was correlated with leptin. There was a significant correlation in group 1 between BMI and age, and in group 3 between acyl-estrone and age (data not shown).

DISCUSSION

The criteria used for classification of the subjects into three groups include the widely accepted criterion that normality in body weight extends up to BMI values of 27 kg/m^2 (24), this figure marking the border between overweight and (mild) obesity. The other criteria used: glycaemia higher than 5.5 mM and insulinaemia higher than 0.2 mM, were selected on the basis of values within the "normal" range, but having a high probability of altered glucose metabolism in predisposed populations (30). The aim was to discriminate not between well-defined situations but between trends, so that the group identified as having a somewhat altered insulin-glucose relationship (group 3) would be discriminated by both markers; this would leave the remaining cases –in which the tendency was not as markedly consistent– in an intermediate group (group 2). Applying the same criteria to the non-obese subjects, a group evolved in which the low glucose and low insulin criteria were not meet as in the controls (group 1) and was discarded because it was too small (N=6, 2 of which were anorexic) and wide dispersion of data. This pruning of controls helped to make the control group 1 much more consistent. The application of essentially metabolic parameters as discriminating factors thus resulted in the

establishment of three groups which, curiously, showed marked differences in weight, fat-free and fat mass. This already points towards a well-established trend: the close relationship between the control of energy handling –including fat deposition– and insulin functionality (1,5).

There was a clear trend towards higher cholesterol, glucose and uric acid levels, all indicators of a deteriorating overall metabolic control in group 3, and to a lesser extent in group 2. The higher levels of aspartate transaminase found in the obese also point in the same direction. The presence of high lactate levels in the group having higher fat mass (group 3) is consistent with the high sensitivity of adipose tissue glycolysis to adrenergic stimulation (31), which results in higher lactate production by this tissue, another factor directly related to high fat content (32).

There is a direct relationship between body fat and circulating levels of acyl-esterified estrone in lean and mildly obese humans of both sexes (23). This relationship, however, is limited to the non-obese as found here, since in most of the obese women studied, the circulating levels of acyl-estrone were lower than the values expected from the relationship observed in the non-obese, and the correlation of acyl-estrone with BMI was lost as a consequence of obesity.

Zucker fa/fa rats show plasma acyl-estrone levels similar to those of lean rats (22), but the administration of oleoyl-estrone results in a significant mobilization of fat with no changes in plasma parameters (19,21). Since the synthesis of oleoyl-estrone by adipose tissue is controlled by leptin (16) and oleoyl-estrone affects the expression of the Ob gene (21) it may be assumed that in these rats the alteration of leptin function lowers oleoyl-estrone levels. Oleoyl-estrone has also been found to modify the ponderostat setting in rats, which strengthens its postulated role as a ponderostat signal (16). The low-for-weight circulating acyl-estrone levels in obese women is similar to that found in genetically obese rats. These data are consistent with an altered function of acyl-estrone in obesity.

The case of acyl-estrone contrasts with that of leptin. Leptin levels were much higher in the obese than in the nonobese women, as previously observed (9,10), again in parallel to the case of Zucker fa/fa rats (33). We found a clear correlation between leptin and the parameters that determine fatness: BMI and percentage of fat. The correlations between leptin and fatness indicators were mostly maintained in the obese groups, suggesting that in women, leptin synthesis –and possibly function– was not significantly altered except in morbid obesity; this is consistent with the lack of changes in leptin levels found in obese women after these levels are adjusted for age and fat mass (unpublished). This is further supported by the unaltered leptin handling (34) and the relative scarcity of mutations in the leptin gene or its receptor (35-39) in humans. Insulin enhances the synthesis and release of leptin in humans (40,41), but the association between hyperinsulinemia and leptin is lost in obesity (42). In this study we observed, however, that although leptin levels were high in obese women, they were uncorrelated with those of insulin except for group 3, in which the alterations in insulin were more marked.

Insulin changes, practically intrinsic to obesity, have often been considered to be a consequence of the metabolic alterations of obesity rather than a key factor in the development of this condition (43).

The data presented here show that early / mild insulin alteration is consistent with obesity, and suggest that the intensity of alteration in insulin function –i.e. insulin resistance– is a key factor defining severe / complicated obesity. The correlation found in the severe obesity group (group 3) between insulin levels and those of leptin, and the low circulating acyl-estrone levels suggest that the influence of insulin on the signals that control the adjustment of body weight may be much more significant than has usually been assumed. Leptin (44,45) and oleoyl-estrone (46) are known to affect insulin levels and resistance, but their interrelationship during obesity is far from being fully understood.

In conclusion, we have found that in obese women, acyl-estrone levels –and probably function– are diminished, contrasting with maintained leptin-BMI correlations. Insulin role in the control of body weight, perhaps through acyl estrone-mediated effects, should be re-evaluated.

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REFERENCES

1 Alemany M. 1989 The etiologic basis for the classification of obesity. Progr Food Nutr Sci 13: 43-66.

2 Walker M. 1995 Obesity, insulin resistance, and its link to non-insulin- dependent diabetes mellitus. Metabolism 44: (9 Suppl. 3)18-20.

3 Cuatrecasas G, Granada ML, Formiguera X, Rull M, Alastrué A, Remesar X, Alemany M, Foz M. 1998 Increased leptin production in vivo and insulin cleavage by the omental adipose tissue of morbidly obese patients. Clin Endocrinol 48: 181-185.

4 Meylan M, Felber JP, Henny C, Jequier E, Temler E. 1987 Metabolic Factors in the Insulin Resistance in Human Obesity. Metabolism 36: 256-261.

5 Cusin I, Rohner-Jeanrenaud F, Terrettaz J, Jeanrenaud B.1992 Hyperinsulinemia and its Impact on Obesity and Insulin Resistance. Int J Obesity 16: (suppl. 4): S1-S11.

6 Schrezenmeir J.1996 Hyperinsulinemia, hyperproinsulinemia and insulin resistance in the metabolicsyndrome. Experientia 52: 426-432.

7 Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. 1994 Positional cloning of the mouse obese gene and its human analogue. Nature 72: 425-432.

8 Halaas JL, Gajiwala KS, Maffei M, Cohen SL, Chait BT, Rabinowitz D, Lallone RL, Burley SK, Friedman JM. 1995 Weight-reducing effects of the plasma protein encoded by the obese gene. Science 269: 543-546.

9 Dagogo-Jack S, Fanelli C, Paramore D, Brothers J, Landt M. 1996 Plasma leptin and insulin relationships in obese and nonobese humans. Diabetes 45: 695-698.

10 Pijl H, Toornvliet AC, Meinders AE. 1996 Serum leptin in normal-weight and obese humans. N Engl J Med 334 :1544.

11 Clément K, Lahlou N, Ruiz J, Hager J, Bougnères P, Basdevant A, Guy-Grand B, Froguel P. 1997 Association of poorly controlled diabetes with low serum leptin in morbid obesity. Int J Obesity 21: 556-561.

12 Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T, Collins F. 1995 Effects of the obese gene product on body weight regulation in ob/ob mice. Science 269: 540-543.

13 Chua SC, Chung W.K, Wu-Peng X.S, 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.

14 Considine RV, Caro J.F. 1997 Leptin and the regulation of body weight. International J Biochem Cell Biol 29: 1255-1272.

15 Kieffer TJ, Keller RS, Leech CA, Holz GG, Habener JF. 1997 Leptin suppression of insulin secretion by the activation of ATP- sensitive K+ channels in pancreatic b-cells. Diabetes 46: 1087-1093.

16 Esteve M, Virgili J, Aguilar H, Balada F, Fernández-López JA, Remesar X, Alemany M. 1999 Leptin enhances the synthesis of oleoyl-estrone from estrone in white adipose tissue. Eur J Nutr 38: 99-104.

17 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.

18 Balada F, Sanchis D, Virgili J, Grasa MM, Monserrat C, Fernández-López JA, 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.

19 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.

20 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.

21 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.

22 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 affects the ponderostat setting differently in lean and in obese Zucker rats. Int J Obesity 22: 366-373.

23 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 (Oxford) 50: 253-260.

24 Aranceta J, Barbany M, Bellido D, Carrillo M, Durán S, Fernández-Soto ML, Formiguera X, Foz M, García-Luna PR, Griera JL, Martínez Valls JF, Morejón E, Moreno B, Serra Majem L, Serrano Ríos M, Tebar FJ. 1996 Consenso español 1995 para la evaluación de la obesidad y para la realización de estudios espidemiológicos. Med Clin (Barcelona) 107: 782-787.

25 Valtueña S, Salas-Salvadó J, Lorda PG. 1997 The respiratory quotient as a prognostic factor in weight-loss rebound. Int J Obesity 21: 267-273.

26 Bioelectrical Impedance Analysis in Body Composition Measurement. 1996 NIH Technol Assess Statement 1994 Dec 12-14. Am J Clin Nutr 64: 524S-532S.

27 Segal KR, Van Loan M, Fitzgerald PI, Hodgdon JA, Van Itallie TB. 1998 Lean body mass estimation by bioelectrical impedance analysis: a four-site cross-validation study. Am J Clin Nutr 47: 7-14.

28 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.

29 Gutmann I, Wahlefeld AW.1974 L-(+)-lactate. In: Bergmeyer HU (ed) Methods of Enzymatic Analysis. Academic Press, New York . pp. 1464-1468.

30 Costa A, Rios M, Fernandez M, Gomis R, Conget I. 1999 The new 1997 ADA diabetes diagnostic categories: impact on employees' annual medical examination. Diabetes Medicine in the press.

31 Faintrenie G, Géloën A. 1996 Alpha-1 adrenergic regulation of lactate production by white adipocytes. J Pharmacol Exp Therap 277: 235-238.

32 Jansson PA, Smith U, Lonnroth P. 1990 Evidence for lactate production by human adipose-tissue in vivo. Diabetologia 33: 253-256.

33 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.

34 Lönnqvist F, Nordfors L, Jansson M, Thörne A, Schalling M, Arner P. 1997 Leptin secretion from adipose tissue in women - Relationship to plasma levels and gene expression. J Clin Invest 99: 2398-2404.

35 Maffei M, Stoffel M, Barone M, Moon B, Dammerman M, Ravussin E, Bogardus C, Ludwig DS, Flier JS, Talley M, Auerbach S, Friedman JM. 1996 Absence of mutations in the human OB gene in obese diabetic subjects. Diabetes 45: 679-682.

36 Matsuoka N, Ogawa Y, Hosoda K, Matsuda J, Masuzaki H, Miyawaki T, Azuma N, Natsui K, Nishimura H, Yoshimasa Y, Nishi S, Thompson DB, Nakao K. 1997 Human leptin receptor gene in obese Japanese subjects: evidence against either obesity-causing mutations or association of sequence variants with obesity. Diabetologia 40: 1204-1210.

37 Considine RV, Considine EL, Williams CJ, Hyde TM, Caro JF. 1996 The hypothalamic leptin receptor in humans - Identification of incidental sequence polymorphisms and absence of the db/db mouse and fa/fa rat mutations. Diabetes 45: 992-994.

38 Clément K, Vaisse C, Lahlou N, Cabrol S, Pelloux V, Cassuto D, Gourmelen M, Dina C, Chambaz J, Lacorte JM, Basdevant A, Bougneres P, Lebouc Y, Froguel P, Guy-Grand B. 1998

A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. Nature 392: 398-401.

39 Strobel A, Issad T, Camoin L, Ozata M, Strosberg AD. 1998 A leptin missense mutation associated with hypogonadism and morbid obesity. Nature Gen 18: 213-215.

40 Leroy P, Dessolin S, Villageois P, Moon BC, Friedman JM, Ailhaud G, Dani C. 1996 Expression of ob gene in adipose cells - Regulation by insulin. J Biol Chem 271: 2365-2368.

41 Barr VA, Malide D, Zarnowski MJ, Taylor SI, Cushman SW. 1997 Insulin stimulates both leptin secretion and production by rat white adipose tissue. Endocrinology 138: 4463-4472.

42 Kim-Motoyama H, Yamaguchi T, Katakura T, Miura M, Ohashi Y, Yazaki Y, Kadawaki T. 1997 Serum leptin levels are associated with hyperinsulinemia independent of body mass index but not with visceral obesity. Biochem Biophys Res Commun 239: 340-344.

43 Bodkin NL, Nicolson M, Ortmeyer HK, Hansen BC. 1996 Hyperleptinemia: Relationship to adiposity and insulin resistance in the spontaneously obese rhesus monkey. Horm Metabol Res 28: 674-678.

44 Remesar X, Rafecas I, Fernández-López JA, Alemany M. 1997 Is leptin an insulin counter-regulatory hormone?. FEBS Lett 402: 9-12.

45 Adán C, Vilà R, Grasa MM, Esteve M, Cabot C, Rafecas I, Estruch J, Remesar X, Fernández-López JA, Alemany M, 1997 Oleoyl-estrone treatment improves the Zucker fa/fa rat resistance to insulin. Int J Obesity 21 (suppl.2): S111(abstract).