Serum Leptin in Obese Women With Polycystic Ovary Syndrome Is Correlated With Body Weight and Fat Distribution But Not With Androgen and Insulin Levels

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Leptin is a hormone produced in the adipose tissue and its concentrations in peripheral blood are significantly correlated with the amount of body fat. Whether other factors, including the pattern of body fat distribution and several hormones (such as insulin, sex steroids, and glucocorticoids), may be involved in the regulation of circulating blood leptin levels is controversial. Women with the polycystic ovary syndrome (PCOS) are hyperandrogenic and most of them are characterized by hyperinsulinemia, insulin resistance, and obesity, particularly the visceral phenotype. To assess the potential contribution of anthropometric factors, androgens, and insulin in determining leptin levels, we examined their relationship with body-mass index (BMI), visceral (VAT) and subcutaneous (SAT) adipose tissue areas, basal androgen levels, and fasting and glucose-stimulated (AUC) insulin in different groups of obese women with PCOS (n = 23) and of age-matched obese (n = 16) and non-obese (n = 10) otherwise healthy controls. The VAT/SAT ratio was measured as a parameter of body fat distribution. Serum leptin levels were significantly higher in obese PCOS women than in obese and normal-weight healthy controls and, within the controls, in the obese than in the non-obese group. In all women considered together, and in each group separately, leptin concentrations were highly significantly correlated with BMI. In addition, after adjusting for BMI, both VAT and the VAT/SAT ratio were positively and significantly correlated with leptin. Partial correlations with the VAT/SAT ratio remained significant in both the obese PCOS group and in controls considered separately, whereas the correlation with the SAT value was significant only in the control group. After adjusting for BMI, no correlation between leptin, androgens and fasting or stimulated (like AUC) insulin was found. These findings indicate that leptin levels in obese women with PCOS are higher than those observed in obese and non-obese controls. Moreover, they suggest that, other than BMI, the pattern of body fat distribution may be an independent factor related to circulating leptin levels, which, on the contrary, do not appear to be related to either androgen or insulin concentrations.

LEPTIN IS A PROTEIN encoded by the *ob* gene, expressed in the adipose tissue, and is thought to be the afferent satiety signal from the adipose mass to the CNS.[1,2] Leptin has been implicated in the regulation of food intake and energy balance.[3,4] In the fed state, circulating leptin concentrations reflect the magnitude of fat stores,[5,6] and leptin levels are elevated in many models of animal obesity and in obese humans, correlating strongly with the degree of obesity.[5,7,8]

Preliminary data obtained in rodents indicate that leptin may also be involved in the regulation of gonadal function and fertility.[8] In fact, injection of leptin in *ob/ob* mice, which are characterized by severe obesity, insulin resistance, and infertilty, increases the levels of gonadotropin, promotes ovarian follicular development, and restores fertility.[9] Women with polycystic ovary syndrome (PCOS) are frequently obese (particularly those with abdominal phenotype), insulin-resistant and hyperinsulinemic, and infertile.[10,11] There is controversy as to whether leptin levels are abnormal in women with PCOS. Although some investigators[12] found that PCOS women have higher leptin levels than their nonaffected counterparts, either obese or normal-weight, other studies did not confirm these findings.[13,14] In addition, some[12] but not all investigators[13,15,16] found that leptin concentrations were significantly correlated with serum androgens and insulin, regardless of their relationship with body weight and total body fat.

The aim of this study was to investigate the relationship between leptin, body weight and fat distribution, and peripheral insulin and androgen concentrations of obese women with PCOS and in age-matched groups of obese and normal-weight otherwise healthy controls.

MATERIALS AND METHODS

Subjects

Three groups of women were enrolled into the study: 23 obese women with PCOS (ob-PCOS), 16 obese (body-mass index [BMI] > 28 kg/m^2) women, and 10 normal-weight women (BMI < 26) who served as controls. All PCOS and obese control women had been referred to the Endocrine Unit of the Department of Internal Medicine and Gastroenterology, University of Bologna, as outpatients for evaluation and treatment of obesity and/or PCOS. Normal-weight controls were selected from doctors and nurses of the staff. All subjects gave written and informed consent to the protocol study.

The diagnosis of PCOS was performed based on the coexistence of oligomenorrhea (<10 menstrual cycles per year) or amenorrhea (absence of menstrual cycle for 3 months), increased blood androgen concentrations, and ultrasound examination of the ovaries, according to previously described criteria.[17] Hyperandrogenism was defined by supranormal blood testosterone and androstenedione and/or dehydroepiandrosterone sulfate levels. None of the women had altered thyroid function, diabetes, or other endocrine dysfunctions, or relevant abnormalities of liver, kidney, and cardiovascular function by routine examination. In particular, Cushing's syndrome, androgen-secreting tumors, and late-onset adrenal hyperplasia were excluded by a standard overnight 1-mg dexamethasone-suppression test and Corthrosyn- (Organon, Westbury, NY, USA) stimulated androgen levels.

None took drugs for at least 1 month before the study, nor were any dieting.

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Anthropometry

Body height was measured without shoes to the nearest 0.5 cm, and body weight without clothes. The waist and hip circumferences (WHR) were measured with the subjects standing, using a 1-cm wide measuring tape, according to the following procedure: waist circumference was obtained as the minimum value between the iliac crest and the lateral costal margin, whereas hip circumference was determined as the maximum value over the buttocks as previously described,[17] and their ratio was calculated to define different patterns of body fat distribution. Body fat distribution was also defined by computed tomography (at the L4-L5 level) according to Sjostrom,[18] and total adipose tissue area, visceral adipose tissue (VAT), area, subcutaneous adipose tissue (SAT) area, and their ratios (VAT/SAT) were calculated. General characteristics and anthropometric data of PCOS women and the control groups are listed in Table 1.

Hormones and Biochemistry

Control women were examined in the follicular phase of the menstrual cycle and, in any case, never more than 10 days after the start of the previous menstrual cycle, whereas obese PCOS women were examined in the early follicular phase of the menstrual cycle if they had mild to moderate oligomenorrhea, or randomly if severe oligomenorrhea or amenorrhea was present. Before testing, all women followed their usual diet, provided at least 250 to 300 g of carbohydrate were ingested. Blood tests were obtained in the morning (8 to 9 AM) after overnight fasting, while subjects had been quietly lying for at least 5 to 10 minutes after an indwelling catheter had been placed in an antecubital vein of an arm kept patent with slowly infused normal saline. Blood samples for leptin, androstenedione, testosterone, dehydroepiandrosterone sulfate, and sex hormone–binding protein (SHBG) were drawn in basal conditions. Moreover, each woman underwent an oral glucose (75 g; Curvosio, Sclavo, Italy) tolerance test (OGTT), taking blood samples for glucose determination in basal conditions and after 30, 60, 90, 120, and 180 minutes, and for insulin determination in basal conditions and after 60, 120, and 180 minutes.

Immediately after the blood samples were taken, they were placed in different tubes for hormone determinations and stored at −20°C until assayed. All assays of each woman were performed in duplicate. Androgen concentrations were assayed as previously described.[11] The SHBG level was measured by a noncompetitive liquid-phase immunoradiometric assay, using a ^{125}I-labeled monoclonal SHBG antibody and an anti-SHBG antiserum with reagents obtained from Pharmos Diagnostic (Ounlunsalo, Finland). Plasma leptin was measured by radioimmunoassay (Linco Research, St Charles, MO). The components of the kit included recombinant human leptin, rabbit antiserum against human leptin, and quality-control pools. In brief, 100-mL aliquots of standards (0, 0.5, 1, 2, 5, 10, 20, 50, and 100 ng/mL) or samples were incubated overnight at 4°C with primary antibody and 12,000 cpm of radioiodinated human leptin. The assay volume was 0.3 mL. Bound and unbound tracer were separated with a second antibody against rabbit immunoglobulin by centrifugation after an additional 20-minute incubation at 4°C. The pellet was then counted and standard curves were calibrated. Maximal tracer binding was approximately 47% and half-maximal binding occurred at 4.3 ng/mL unlabeled leptin. Sensitivity was 0.5 ng/mL and the intraassay and interassay coefficients of variation were less than 3% and less than 10%, respectively.

Table 1. General Characteristics (mean ± SD) and Anthropometric Parameters of Obese PCOS Women and of the Two Control Groups

Parameters	Obese PCOS (n = 23)	Controls	
		Normal-Weight (n = 10)	Obese (n = 16)
Age (yr)	29.7 ± 4.1	26.4 ± 4.9	30.1 ± 7.2
Body weight (kg)	97.0 ± 19.1*	54.0 ± 5.4	101.9 ± 19.3‡
BMI (kg/m^2)	37.2 ± 7.9*	21.2 ± 0.9	39.0 ± 7.5‡
WHR	0.86 ± 0.07*	0.71 ± 0.04	0.83 ± 0.10†
VAT (cm^2)	129 ± 74* (13)	21 ± 8 (6)	126 ± 77‡ (15)
SAT (cm^2)	498 ± 123* (13)	120 ± 78 (6)	573 ± 119‡ (15)
VAT/SAT ratio	0.26 ± 0.15 (13)	0.21 ± 0.08 (6)	0.21 ± 0.12 (15)

NOTE. Numbers in parentheses refer to the number of observations.

*$P < .01$ for obese PCOS *v* normal-weight controls.

†$P < .05$ and ‡$P < .01$ for obese *v* normal-weight controls.

Statistics

Results are expressed as means ± SD. During the OGTT, insulin and glucose areas under the curve (AUC) were calculated by the trapezoidal method. Intergroup comparisons were performed by means of the Mann-Whitney nonparametric *U* test, and the ANCOVA, to control for the effects of several variables (BMI and VAT/SAT ratio) on serum leptin concentrations. Simple (Pearson) and multiple regression analysis were also used to evaluate the correlations between the variables. *P* less than .05 was used to define statistical significance.

RESULTS

Anthropometry

There were no significant differences in age values between ob-PCOS and the control groups. Both ob-PCOS and obese controls had similar BMI, WHR, SAT, and VAT values; however, values were significantly higher than in normal-weight controls. There was no significant difference in the VAT/SAT ratio between the groups, although higher mean levels were found in the ob-PCOS women (Table 1).

Leptin, Sex Steroids, and Insulin

Leptin concentrations were significantly higher in ob-PCOS group than in obese and normal-weight control groups ($P < .01$ and $P < .05$, respectively) and within the controls in the obese group than in normal-weight group ($P < .05$). Testosterone and androstenedione concentrations were significantly higher in the ob-PCOS group ($P < .01$) than in the other groups, while SHBG levels were not significantly different in ob-PCOS and obese control groups, but were lower ($P < .05$) than in the normal-weight control group. Three women (17%) in the ob-PCOS group and four of the obese controls had impaired glucose tolerance based on the National Diabetes Data Group criteria.[20] There were no significant differences among the three groups in the fasting and glucose-stimulated (like AUC) glucose concentrations; on the contrary, both fasting and insulin AUCs were significantly higher in the obese groups than in normal-weight controls ($P < .05$), whereas no significant difference was present in insulin values between ob-PCOS and obese controls (Table 2).

Relationship Between Leptin, Anthropometry, and Hormones

In the whole group of women, including ob-PCOS and controls, leptin concentrations were significantly correlated with body weight ($r = .69$; $P < .001$) and BMI ($r = .69$; $P < .05$). Partial correlation coefficients between leptin and indices of body fat distribution are listed in Table 3. Taking ob-PCOS and controls as a group, and after adjusting for BMI, both VAT and the VAT/SAT ratio, but not SAT, were positively and significantly correlated with leptin levels. Partial correla-

Table 2. Hormones and Metabolic Parameters (mean ± SD) in Obese PCOS Women and in the Two Control Groups

Parameters	Obese PCOS (n = 23)	Controls	
		Normal Weight (n = 10)	Obese (n = 16)
Leptin (ng/mL)†	49.8 ± 27.0[b,c]	5.9 ± 4.3	33.0 ± 13.0[e] (15)
Testosterone (nmol/L)	2.71 ± 1.10[b,d]	1.44 ± 0.50	1.70 ± 0.65 (10)
Androstenedione (nmol/L)	9.7 ± 4.0[b,d]	5.2 ± 0.9 (9)	5.6 ± 2.0 (12)
DHEA-S (nmol/L)	1,657 ± 703	2,766 ± 1,350 (9)	2,342 ± 1,305 (13)
SHBG (nmol/L)	16.6 ± 9.4[b]	61.2 ± 18.3 (5)	26.7 ± 14.0[e]
Fasting glucose (nmol/L)	98.9 ± 26.2	85.7 ± 10.8	96.3 ± 14.1
Fasting insulin (pmol/L)	440 ± 917[b] *	50.1 ± 29.6	180 ± 104[e]
$Glucose_{AUC}$ (nmol/L/min^{-1})	19,880 ± 6,414	18,455 ± 3,047 (9)	18,621 ± 3,977
$Insulin_{AUC}$ (pmol/L/min^{-1})	268,157 ± 313,153[a,c] (22)	59,674 ± 27,466 (8)	132,531 ± 29,528

NOTE. Numbers in parentheses refer to the number of observations.

*The very high SD value was due to a woman presenting fasting insulin of 4,456 pmol/L by excluding this woman, the mean values of the group was still significantly different vs controls.

[a]$P < .05$ and [b]$P < .01$ for obese PCOS *v* normal-weight controls; [c]$P < .05$ and [d]$P < .01$ for obese PCOS *v* obese controls; [e]$P < .01$ for obese *v* normal-weight controls.

†To control for the effects of BMI and VAT/SAT ratio on leptin levels, the ANCOVA was applied: $F = 9.62$, $P = .004$ (BMI: $F = 20.69$, $P = .0001$; VAT/SAT ratio, $F = 5.86$; $P = .022$).

tions with the VAT/SAT ratio remained significant in both ob-PCOS and controls considered separately, whereas the correlation with the SAT value was significant only in the control group (Fig 1).

After adjusting for BMI, no correlations between leptin and fasting and stimulated insulin, testosterone, androstenedione, DHEA-S, and SHBG were found.

DISCUSSION

This study shows that leptin levels in ob-PCOS were significantly higher than in controls, both obese and non-obese, as previously reported by others.[11,15] Theoretically, factors that influence leptin levels in PCOS may be different from those that regulate leptin levels in nonaffected women. Moreover, we have confirmed that leptin is highly significantly correlated to BMI

Table 3. Partial Correlation Coefficients Between Serum Leptin and Parameters of Body Fat Distribution in Obese PCOS and Control Women Taken Together and Separately

Groups	Independent Variables	Total *r*	*P* Value	*T* Value	Partial *P* Value
All		.740	<.001		
	BMI			5.230	<.001
	VAT			2.375	<.03
	BMI			0.692	<.001
	SAT			0.871	NS
	BMI			5.935	<.001
	VAT/SAT ratio			2.110	<.04
ob-PCOS		.716	<.03		
	BMI			3.099	<.02
	VAT			1.550	NS
	BMI			0.710	<.02
	SAT			1.482	NS
	BMI			3.599	<.01
	VAT/SAT ratio			1.969	<.05
Controls		.769	<.001		
	BMI			4.084	<.001
	VAT			1.796	NS
	BMI			0.823	<.001
	SAT			2.988	<.01
	BMI			5.493	<.001
	VAT/SAT ratio			0.327	<.04

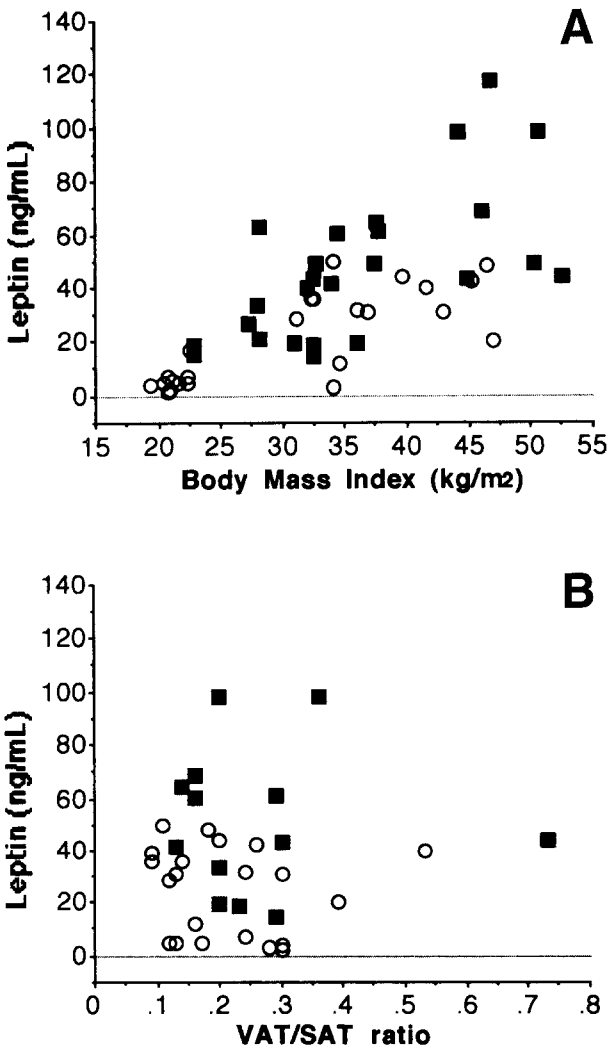

Fig 1. Simple correlation coefficients between leptin, BMI (A) (all: $r = .69$, $P < .001$; obese PCOS: $r = .59$, $P < .01$; controls: $r = .76$, $P < .01$), and the VAT/SAT ratio (B) (all: $r = .04$, P = not significant [NS]; obese PCOS: $r = .03$, P = NS; controls: $r = .09$, P = NS) in obese PCOS women (■) and in age-matched obese and normal-weight controls (○).

not only in normal-weight and obese otherwise healthy women, but also in PCOS women. Therefore, the amount of total fat appears to be the major determinant of leptin in both controls and PCOS.[5,7] However, after accounting for the effects of BMI, we found an independent positive association between circulating leptin levels and the VAT and the VAT/SAT ratio values, which suggests that leptin may be indepently related to the amount of visceral fat not only in nonhyperandrogenic women with normal weight or obesity, but also in obese women with PCOS. This is not surprising, since it has been shown that leptin mRNA expression is higher in visceral than subcutaneous fat.[21] Previous studies of our group have demonstrated that a great proportion of women with PCOS may have high values of the WHR, independent of BMI, and therefore, predominant abdominal fat distribution,[11] and that this anthropometric pattern may be significantly correlated to both prevailing hyperinsulinemia and excess androgens levels.[11] Whether leptin concentrations are related to body fat distribution in women with and without hyperandrogenism is a matter of controversy. In fact, both positive[16] and negative studies[12] have been reported in women with PCOS. Our data indicate that the relationship between leptin and VAT/SAT ratio persists in women, regardless of whether they have PCOS. Therefore, the amount of abdominal fat may be a factor influencing leptin levels in peripheral blood both in women with PCOS and in nonaffected controls. The fact that women with PCOS are frequently characterized by abdominal fat distribution much more than nonhyperandrogenic women, regardless of whether they are obese or normal-weight,[11] could contribute to explain the higher than normal leptin values that we and others have found.[12]

However, this finding needs to be confirmed in a larger cohort of women with and without hyperandrogenic state. On the other hand, it seems to be supported also by the fact that the same relationship has been reported by Haffner et al[22] in a large group of unselected men ranging from normality to the obese state. Several other factors, including insulin, glucocorticoids, and, possibly, sex steroids, have been adovocated to regulate the expression of the *ob* gene in the adipose tissue.[2,23] The relationship between leptin and fasting or glucose-stimulated insulin levels in PCOS is controversial. However, although a positive influence of insulin on fasting leptin levels has been reported,[15] in most of the studies performed in PCOS,[12,13,16] a convincing relationship between insulin and leptin has not emerged. On the other hand, this is in agreement with what is reported in other studies performed in obese and non-obese nonhyperandrogenic women.[24-26] Therefore, whether insulin may be a regulatory factor of leptin concentrations in vivo remains controversial and requires further more detailed studies.

Most of the studies published so far have been performed in PCOS women, since they may represent a good model for studying the androgen-leptin interaction in humans. This concept originally emerged from several findings confirming that females have higher leptin levels than males.[7] In fact, although this difference could be accounted for, at least in part, by the higher fat mass in women than in men, some investigators suggest that this sexual dimorphism will be explained by the effect of androgens.[27,28] Unfortunately, studies on the effects of sex steroids on leptin mRNA expression and function are lacking. In women with PCOS, several studies failed to observe any significant correlation between leptin and total or free testosterone levels,[13,16] although Brzechffa et al[12] found that a subset of PCOS women with very high leptin levels also had free testosterone concentrations higher than those with leptin levels included in the normal range. Taken together, these preliminary data and our own make it unlikely (although they do not exclude it) that high androgen levels may be responsible for higher than expected (on the basis of BMI values) leptin levels in obese PCOS women.

In summary, in this study we have found that (1) women with obesity and PCOS have higher leptin levels than normal weight and weight-matched obese controls; (2) BMI and the pattern of body fat distribution are independently correlated with leptin in both obese PCOS and controls, and finally (3) no relationship was present between leptin, fasting and glucose-stimulated insulin levels, and androgens.

REFERENCES

1. Campfield LA, Smith FJ, Guisez Y, et al: Recombinant mouse OB protein: Evidence for a peripheral signal linking adiposity and central neural networks. Science 269:546-549, 1995
2. Rohner-Jeanrenaud F, Jeanreaud B: Obesity, leptin and the brain. N Engl J Med 334:324-325, 1996
3. Rink TJ: In search of a satiety factor. Nature 327:406-407, 1995
4. Frederich RC, Hamann A, Anderson S, et al: Leptin reflect blood lipid content in mice: Evidence for diet-induced leptin resistance. Nat Med 1:1311-1314, 1995
5. Maffei M, Halaas J, Ravussin E, et al: Leptin levels in human and rodent: Measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. Nat Med 1:1155-1161, 1995
6. Frederich RC, Lollmann B, Hamann A, et al: Expression of ob mRNA and its encoded protein in rodents. Impact of nutrition and obesity. J Clin Invest 96:1658-1663, 1995
7. Considine RV, Sinha MK, Heiman HL, et al: Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N Engl J Med 334:292-295, 1996
8. Chebab F, Lim M, Lu R: Correction of the sterility defect in homozygous obese female mice by treatment with the human recombinant leptin. Nat Genet 12:318-320, 1996
9. Barash IA, Cheung CC, Weigle DS, et al: Leptin is a metabolic signal to the reproductive system. Endocrinology 137:3144-3147, 1996
10. Franks S: Polycystic ovary syndrome. N Engl J Med 333:853-861, 1995
11. Pasquali R, Casimirri F, Venturoli S, et al: Body fat distribution has weight-independent effects on clinical, hormonal, and metabolic features of women with polycystic ovary syndrome. Metabolism 43:706-713, 1994
12. Brzechffa PR, Jakimiuk AJ, Agarwal SK, et al: Serum immunoreactive leptin concentrations in women with polycystic ovary syndrome. J Clin Endocrinol Metab 81:4166-4169, 1996
13. Mantzoros C, Dunaif A, Flier JS: Leptin concentrations in the polycystic ovary syndrome. J Clin Endocrinol Metab 82:1687-1691, 1997
14. Rouru J, Anttila L, Koskinen P, et al: Serum leptin concentrations in women with polycystic ovary syndrome. J Clin Endocrinol Metab 82:1697-1700, 1997
15. Laughlin GA, Morales AJ, Yen SS: Serum leptin levels in women with polycystic ovary syndrome: The role of insulin resistance/hyperinsulinemia. J Clin Endocrinol Metab 82:1692-1696, 1997
16. Chapman IM, Wittert GA, Norman RJ: Circulating leptin

concentrations in polycystic ovary syndrome: Relation to anthropometric and metabolic parameters. Clin Endocrinol (Oxf) 46:175-181, 1997

17. Orsini LF, Venturoli S, Lorusso L, et al: Ultrasonic findings in polycystic ovarian disease. Fertil Steril 43:709-714, 1985

18. WHO: Measuring Obesity. Classification and description of anthropometric data. Copenhagen, Denmark, World Health Organization, Regional Office for Europe, Eur/ICP/NUT 125-0612v, 1988

19. Sjostrom L: A computer-tomography based multicompartment body composition technique and anthropometric predictions of lean body mass, total and subcutaneous adipose tissue. Int J Obes 15:19-30, 1991 (suppl 2)

20. National Diabetes Data Group: Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. Diabetes 28:1039-1057, 1979

21. Masuzaki H, Ogawa Y, Isse N, et al: Adipocyte-specific expression and regional differences in the adipose tissue. Diabetes 44:855-858, 1997

22. Haffner SM, Miettinen H, Karhapa P, et al: Leptin concentrations, sex hormones, and cortisol in non diabetic men. J Clin Endocrinol Metab 82:1807-1809, 1997

23. Saladin R, De Vos P, Guerre-Millo R, et al: Transient increase in obese gene expression after food intake or insulin administration. Nature 377:527-529, 1995

24. Segal RK, Landt M, Klein S: Relationship between insulin sensitivity and plasma leptin concentration in lean and obese men. Diabetes 45:988-991, 1996

25. Dagogo-Jack S, Fanelli C, Paramore D, et al: Plasma leptin and insulin relationships in obese and nonobese humans. Diabetes 45:695-698, 1996

26. Kolaczynski JW, Nyce MR, Considine RV, et al: Acute and chronic effect of insulin on leptin productions in humans. Diabetes 45:699-701, 1996

27. Rosenbaum M, Nicolson M, Hirsh J, et al: Effects of gender, body composition, and menopause on plasma concentrations of leptin. J Clin Endocrinol Metab 81:3424-3427, 1996

28. Saad MF, Damani S, Gingerich RL, et al: Sexual dimorphism in plasma leptin concentrations. J Clin Endocrinol Metab 82:579-584, 1997