Serum Leptin Elevation in Obese Women with PCOS: A Continuing Controversy

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Submitted April 9, 2003; accepted July 19, 2004

Purpose: To evaluate leptin levels in a sample of obese women with PCOS and compare the results with obese and non-obese control, to be ultimately correlated with BMI, and insulin sensitivity.

Methods: Leptin and insulin assays by immuno-radiometric method, glucose assay by enzymatic colorimetric method.

Results: Leptin levels were significantly different between obese and non-obese subjects, and were significantly different between insulin resistant and non-insulin resistant obese PCOS, but were not significantly different between obese non-insulin resistant PCOS, and obese controls.

Conclusions: Body mass index and insulin resistance are the two main factors governing serum leptin levels.

KEY WORDS: Body mass index; leptin; obesity; polycystic ovary syndrome.

INTRODUCTION

It is an apparent paradox that leptin which was named after the Greek word for lean is encoded by the obesity gene (*Ob*). This unfortunate misnaming occurred because it is leptin deficiency that was discovered first in obese mice, homozygous for mutation of *Ob* gene (*ob/ob*) with subsequent inability to produce leptin (1). Leptin, a 167 amino acid peptide of tumor necrosis factor family of cytokines (2), is secreted from adipose tissue in pulsatile fashion with nocturnal rise and circulates in body fluids either as free protein or bound to the soluble isoform of its receptor (3). The known function of leptin is regulating energy homeostasis by

influencing feeding behavior and energy expenditure (4,5), so that low fat stores and hypoleptinemia stimulate appetite and reduce thermogenesis while excessive accumulation of body fat is prevented through hyperleptinemia inducing the opposite effect.

The relationship between fat and reproduction has been recognized for over three decades (6). Leptin was only the mysterious missing link in Frisch critical body fat hypothesis (7), which assumes that a critical percent of body fat is required for establishment and maintenance of reproductive function. It is logic to assume that when the available energy is insufficient due to starvation or diverted in exercise; reproduction should be suspended to support essential metabolism for survival (8). What is really intriguing is why excessive accumulation of body fat is also associated with reproductive dysfunction. Although Neel hypothesis (9) of thrifty genotype, is a nice trial to conceptualize the problem, it does not explain the mechanism of dysfunction. On the basis of this hypothesis, it was speculated that from genetic standpoint two populations exist: one with low insulin resistance who reproduce well during the times of plenty, and the

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other with high resistance to insulin who reproduce best during famine. The existence of these two genotypes would help to ensure optimum survival of the species, however harsh or favorable the environment (10).

The concept of leptin resistance (4), was introduced in an apparent analogy with that of insulin resistance and perhaps in association with it, to explain why hyperleptinemia associated with obesity fails to correct the underlying defect in energy homeostasis. Suggested mechanisms of leptin resistance included a defect in the transport mechanism into cerebrospinal fluid (11,12) and/or altered rhythmicity of leptin secretion (13). In polycystic ovary syndrome (PCOS) there is a vicious circle of endocrine dysregulation that can be initiated at any of many entry points (14) but central to this pathogenesis and perhaps a common effector is hyperandrogenism. Androgens appear to have dual effect on the ovary, so, early in folliculogenesis they induce large cohort of follicles to enter the growth trajectory (15), while late in folliculogenesis androgens suppress aromatase activity and cause premature shift of granulosa cells from proliferating to steroidogenic state (16,17). Still, there are some gaps in our understanding of the pathogenesis of PCOS, especially in relation to the characteristic phenotype and gonadotrophic hormone abnormalities seen in these cases. β Endorphin (18), inhibin B (19), follistatin (20), and leptin are candidate mediators that might interconnect these diverse abnormalities.

In this study, serum leptin is evaluated in obese women with PCOS in comparison with obese and nonobese controls, and the results are correlated to body mass index (BMI), fasting insulin levels, insulin glucose ratio (IGR) as an indicator of insulin resistance as well as other reproductive endocrine parameters.

SUBJECTS AND METHODS

This study was performed on 70 women in the reproductive age (20–40 years) classified into three groups:

Group I: consisting of 10 nonobese ovulatory control.
Group II: consisting of 10 obese ovulatory control.
Group III: which is the study group consisting of 50 obese women with polycystic ovary syndrome (PCOS).

Cut-off value for diagnosis of obesity was taken as $\text{BMI} \geq 28 \text{ kg/m}^2$, and diagnosis of PCO was based on

the presence of chronic anovulation and characteristic ovarian morphology seen by vaginal sonography.

Sonographic criteria for diagnosis of polycystic ovary were the presence of multiple (10 or more) small subcortical follicles (3–8 mm in diameter), in addition to enlarged stroma. All women were euglycemic, normo-prolactinemic, and not receiving any hormonal treatment for at least 2 months prior to the study.

All selected women included in this work were subjected to complete history taking with special emphasis on menstrual history, thorough general examination including estimation of body weight and height to calculate the BMI. Vaginal sonography was done to detect PCO morphology.

Blood sampling was done in control group (Groups I and II) during the early follicular phase (3rd–5th menstrual day). In the study group (Group III), because the patients were often amenorrheic, blood samples were withdrawn at random but after sonographic exclusion of dominant follicular activity.

After obtaining fasting blood samples serum was separated, then portion of the sample was used for insulin and glucose estimation and the rest was immediately frozen (-70°C) until hormonal assays were performed.

Fasting serum glucose was determined using an enzymatic colorimetric method. After determination of fasting serum insulin level, insulin/glucose ratio (I/G) was calculated as an index of insulin resistance (IR) (19). Hyperinsulinemia and IR were defined when I/G ratio was $\geq 23 \mu\text{IU/mg}$, taking in consideration the upper normal limit for fasting insulin ($25 \mu\text{IU/mL}$) and for glucose (110 mg/dL).

Assay Procedures

Serum insulin concentrations were measured using an immunoradiometric assay kit for human insulin (Biosource Europe S.A., Belgium). The assay is based on coated tube separation and on the oligoclonal system, in which several monoclonal antibodies directed against distinct epitopes of insulin have been used.

Serum prolactin, FSH and LH were measured using a solid-phase two-site chemi-luminescent enzyme immunometric assay (Immulate system, EURO/DPG Ltd., United Kingdom). The solid phase, a polystyrene bead enclosed within an immutate test unit is coated with a monoclonal antibody specific for prolactin, FSH or LH.

Serum testosterone and dehydro-epiandrosterone concentrations were determined using electro-chemiluminescence immunoassay "ECLIA" based on competition principle for use on Elecsys from Roche Diagnostics Corporation, Indianapolis, USA.

Serum leptin was measured by human leptin immunoradiometric assay (IRMA) kit (Diagnostic system laboratories Inc., Texas, USA). The procedure employs a two-site IRMA principle designed to detect leptin. The IRMA is a noncompetitive assay in which the analyte to be measured is "sandwiched" between two antibodies. The first antibody is immobilized to the inside walls of the tubes. The other antibody is radiolabelled for detection. The analyte present in the unknowns, standards and controls is bound by both the antibodies to form a "Sandwich" complex. Unbound reagents are removed by decanting and washing the tubes.

Statistical Analysis

Data were analyzed using SPSS software. The following tests were used:

1. Analysis of variance test (*F* test) was used to compare between the three groups, then LSD test was used to determine the different groups.
2. Multiple regression analysis to correlate BMI, and insulin resistance with serum leptin.

RESULTS

The mean age, BMI, metabolic parameters (glucose, insulin), and hormonal concentrations (FSH,

LH, testosterone, DHEA, leptin) of the three studied groups are shown in Table I.

The mean age did not differ among the groups. There was no difference in mean BMI between patients with PCOS and their obese controls. Serum fasting glucose levels were significantly higher in patients with PCOS and their obese controls compared to the nonobese control subjects. Insulin levels and insulin glucose ratio were significantly higher in the study group as compared to both control groups.

Serum FSH was significantly lower in the study group as compared to their obese and nonobese control while LH, testosterone, and DHEA levels were significantly higher, a typical endocrine abnormalities of the PCOS. The mean leptin levels were 65.4 ± 23.73 , 50.9 ± 9.82 , and 22.5 ± 2.29 ng/mL in the study group and their obese and nonobese controls respectively. Leptin levels were significantly higher in obese subjects (Groups II and III) as compared to nonobese group (Group I), more importantly serum leptin levels were significantly higher in the study group than their obese control and this deserved further evaluation.

To detect the factors responsible for this finding, the study group was classified into two subgroups, those with insulin resistance and those without, and a correlation was sought between serum leptin levels and insulin resistance.

Patients with insulin resistance had significantly higher leptin levels, but significantly higher BMI too (Table II). After correction for BMI, insulin resistance was found to be a separate strong predictor of leptin level (Table III), further more when noninsulin resistant subgroup was compared to obese control, no significant difference was found between them in leptin levels.

Table I. Mean Age, Anthropometric Measurement, Metabolic and Hormonal Data in Nonobese Ovulatory (GI), Obese Ovulatory (GII), and Obese PCOS women (GIII)

Variable	Group I (<i>n</i> = 10)	Group II (<i>n</i> = 10)	Group III (<i>n</i> = 50)	<i>F</i> test	<i>P</i> value
Age (years)	28.8 ± 3.88	31.2 ± 3.97	28.1 ± 4.61	2.04	0.138 (NS)
BMI (kg/m ²)	24.04 ± 2.37	33.59 ± 1.23	34.84 ± 4.77	27.59* 2,1–3,1	0.000
Glucose (μIU/mL)	77.50 ± 7.04	86.50 ± 9.59	85.96 ± 8.78	4.17* 2,1–3,1	0.002
Insulin (μIU/mL)	5.64 ± 2.24	9.30 ± 5.71	17.45 ± 9.20	11.10* 3,1–3,2	0.000
Insulin/glucose ratio	7.41 ± 3.31	11.22 ± 8.01	20.09 ± 10.24	9.96* 3,1–3,2	0.000
FSH (mIU/mL)	7.12 ± 1.14	6.8 ± 1.73	5.04 ± 1.4	12.29* 3,1–3,2	0.000
LH (mIU/mL)	4.84 ± 1.2	5.14 ± 1.65	8.74 ± 3.4	11.20* 3,1–3,2	0.000
Testosterone (ng/mL)	0.33 ± 0.2	0.49 ± 0.3	1.10 ± 0.8	6.70* 3,1–3,2	0.002
DHEA (ng/dL)	111 ± 22.6	125.7 ± 24.8	252.3 ± 99.9	17.29* 3,1–3,2	0.000
Leptin (ng/mL)	22.54 ± 2.29	50.90 ± 9.82	65.45 ± 23.73	18.57* 2,1–3,1–3,2	0.000

*Significant.

Table II. BMI and Serum Leptin Level in Noninsulin Resistant and Insulin Resistant Obese Women with PCOS

	Noninsulin resistant subgroup (<i>n</i> = 27) mean $\pm$ SD	Insulin resistant subgroup (<i>n</i> = 23) mean $\pm$ SD	<i>t</i>	<i>p</i>
BMI (kg/m ²)	31.56 $\pm$ 3.04	38.69 $\pm$ 3.34	7.88*	0.000
Leptin (ng/mL)	51.0 $\pm$ 10.41	85.47 $\pm$ 16.73	5.72*	0.000

*Significant.

DISCUSSION

The polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of reproductive age. Clinical, and/or biochemical hyperandrogenism and typical ovarian morphology are almost invariable features suggesting that abnormalities in folliculogenesis and steroidogenesis are central to the disorder. In view of the remarkable heterogeneity of the problem, it is conceivable that no coherent model of pathogenesis fits all the cases and that several links are still missing in all of the suggested scenarios. Leptin is suggested to be one of these missing links.

Initial reports suggested that a substantial proportion of women with PCOS have leptin levels that are higher than expected for their BMI (21). Subsequent studies, however, have provided evidence that circulating leptin levels are fully accounted for by the degree of adiposity and BMI compared to matching control subjects (22–25). On the other hand, Conway and Jacobs (26), reported that, for any given body weight, circulating leptin concentrations are lower in women with PCOS than those without, suggesting that neuro-endocrine recognition of obesity may be impaired in such women. These contradictory findings are probably related to incomplete evaluation of the different variables controlling serum leptin, particularly insulin and androgens. Androgens suppress

leptin production, while insulin stimulates it, so the net result would be a balance between the effects of BMI, androgens, and insulin (27).

In this study, leptin levels were significantly higher in obese PCOS women than their obese control despite comparable BMI, and this finding stimulated further search for the factors implicated in this difference. Further subclassification of the study group according to the presence or absence of insulin resistance was done and data were reanalyzed. Significant difference was found between the two subgroups in leptin levels but they differed also significantly in BMI. Multiple regression analysis was done to find out if insulin resistance was an independent factor affecting leptin levels. As expected, insulin resistance and the associated hyperinsulinemia was found to be responsible for significant part of this difference, and noninsulin resistant subgroup was not found to differ significantly from obese control regarding serum leptin levels.

The association between insulin resistance and hyperandrogenism was described, even before the original description of PCOS by Stein and Leventhal in 1935 (28). In their report Achard and Thiers (29) in 1921 described bearded diabetic women and suggested that a relation exists between insulin/glucose homeostasis abnormality and her virilism. Poretsky and Kahin (30) suggested that insulin acts in concert with several paracrine growth factors as a nonpituitary gonadotrophin to modulate several parts of reproductive endocrine system not just ovarian stroma and under normal physiological situations not just pathologies like PCOS.

Insulin resistance might occur due to inherited abnormalities in insulin or insulin receptor genes (31), but in absence of these genetic abnormalities, leptin offers a plausible explanation for obesity related insulin resistance. It has been shown that leptin inhibits insulin binding in isolated rat adipocytes (32), and attenuates several insulin induced activities in hepatocytes (33). Thus obesity, hyperleptinemia, insulin resistance, and hyperinsulinemia further increasing leptin production constitute a self-perpetuating vicious circle of events.

Abundant leptin receptors have been detected in ovarian granulosa and theca cells (34), furthermore, leptin treatment of these cells in vitro, caused significant reduction in its steroid output (35). It is possible that leptin has a dual effect on reproduction and that the major site of action differs according to the circulating levels (3). The positive action of leptin on hypothalamic pituitary level might be a crucial trigger

Table III. Correlation Between Serum Leptin Levels and Both BMI and Insulin Resistance in the Two PCOS Subgroups

Model	Unstandardized coefficients		Standardized coefficients	<i>t</i>	<i>p</i>
	<i>B</i>	Std. error	Beta		
Constant	−53.550	15.542		−3.446	0.001
IR	14.584	4.715	0.301	3.093*	0.003
BMI	3.256	0.484	0.656	6.731*	0.000

Note. The constant is leptin. IR = Insulin resistance.

*Significant.

of puberty and might play a predominant role in conditions with abnormally low plasma concentrations as in subjects with very low BMI. Conversely in obesity the central leptin receptors which are sensitive to extremely low ligand concentrations are protected from hyperleptinemia by saturable transport system of blood brain barrier, where as peripheral leptin receptors are exposed to high ligand concentration with possible negative effects on gonadal steroidogenesis.

A last question to be answered is: why not all obese women develop insulin resistance and why not all insulin resistant women develop PCOS. The answer lies in the genetic make-up, and this holds the notion that it is only through interaction with environment that genes express themselves (36). An example of this genetic susceptibility is the presence of an abnormality of serine-threonine phosphorylation, which is involved in postreceptor transduction of insulin signal (37) as well as posttranslational regulation of 17,20-lyase activity (38). The presence of such an abnormality could predispose to both insulin resistance and abnormal steroidogenesis in presence of other aggravators like hyperleptinemia.

The paradigm for such an approach would be the use of genetic tests in susceptible families to predict such conditions so that the affected person's life style could be modified, and the adverse effects of genetic constitution counteracted.

CONCLUSION

Leptin is a good candidate for playing an important role in the pathogenesis of obesity related insulin resistance, and perhaps in the ovarian dysfunction encountered in obese PCOS.

REFERENCES

1. Zhang Y, Proenca R, Maffei M, *et al.*: Positional cloning of the mouse obese gene and its human homologue. *Nature* 1994;372:425–432
2. Ahima RS, Flier JS: Leptin. *Annu Rev Physiol* 2000;62:413–437
3. Caprio M, Fabbri E, Isidori AM, *et al.*: Leptin in reproduction. *Trends Endocrinol Metab* 2001;12:65–72
4. Caro JF, Sinha MK, Kolaczynski ZW, *et al.*: Leptin: The tale of an obesity gene. *Diabetes* 1996;45:1455–1462
5. Stephens TW, Caro JF: To be lean or not to be lean. Is leptin the answer? *Exp Clin Endocrinol Diabetes* 1998;106:1–15
6. Kennedy GC, Mitra J: Body weight and food intake as initiating factors for puberty. *J Physiol* 1963;166:408–418
7. Frisch RE: Body fat, puberty, and fertility. *Biol Rev* 1984;59:161–188
8. Wade GN, Schneider JE, Li HY: Control of fertility by metabolic cues. *Am J Physiol* 1996;270:E1
9. Neel JV: *Diabetes mellitus*: A thrifty genotype rendered detrimental by progress? *Am J Hum Gen* 1962;14:353–362
10. Fox R: Polycystic ovarian disease and insulin resistance: Pathophysiology and wider health issues. In *Progress in Obstetrics and Gynecology*, J. Studd, (ed), London, Churchill Livingstone, 1997, Vol 11, Ch 19, pp 351–367
11. Caro JF, Kolaczynski JW, Nyce MR, *et al.*: Decreased cerebrospinal fluid/serum leptin ratio in obesity: A possible mechanism for leptin resistance. *Lancet* 1996;348:159–161
12. Schwartz MW, Peskind E, Raskind M, *et al.*: Cerebrospinal fluid leptin levels: Relation to plasma levels and to adiposity in humans. *Nat Med* 1996;2:589–593
13. Saad MF, Riad-Gabriel MG, Khan A: Diurnal and ultradian rhythmicity of plasma leptin: Effect of gender and adiposity. *J Clin Endocrinol Metab* 1998;83:453–459
14. Norman J, Masters SC, Hague W, *et al.*: Metabolic approaches to the subclassification of polycystic ovary syndrome. *Fertil Steril* 1995;63:329–335
15. Louvet JP, Harman SM, Schreiber JR, *et al.*: Evidence for a role of androgens in follicular maturation. *Endocrinology* 1975;97:366–372
16. Anderson E, Lee GY: The effects of DHEA and its metabolites on the PCO condition: Cytogenetic changes of rat granulosa cells in vitro. *Tissue Cell* 1996;28(6):673–685
17. Fauser BC: Observations in favor of normal early follicular development and disturbed dominant follicle selection in PCOS. *Gynecol Endocrinol* 1994;8:75
18. Lanzone A, Fulghesu AM, Fortini A, *et al.*: Effect of opiate receptor blockade on the insulin response to oral glucose load in polycystic ovarian disease. *Hum Reprod* 1991;6:1043–1049
19. Hillier SG: Regulatory functions for inhibin and activin in human ovaries. *J Endocrinol* 1991;131:171–175
20. Mather JP, Moore A, Li RH: Activin, inhibins, and follistatins: Further thoughts on a growing family of regulators. *Proc Soc Exp Biol Med* 1997;215:209–222
21. Brzechaffa PR, Jakimiuk AJ, Agarwal SK, *et al.*: Serum immunoreactive leptin concentrations in women with polycystic ovary syndrome. *J Clin Endocrinol Metab* 1996;4166–4171
22. Chapman IM, Wiltert JA, Norman RJ: Circulating leptin concentrations in polycystic ovary syndrome in relation to anthropometric and metabolic parameters. *Clin Endocrinol (Oxf)* 1997;46:175–179
23. Rouru J, Antilla L, Koskinen P, *et al.*: Serum leptin concentrations in women with PCOS. *J Clin Endocrinol Metab* 1997;82:1697–1702
24. Mantzoros CS, Dunaif A, Flier JS: Leptin concentrations in the polycystic ovary syndrome. *J Clin Endocrinol Metab* 1997;82:1687–1694
25. Telli MH, Yildirim M, Noyan V: Serum leptin levels in patients with polycystic ovary syndrome. *Fertil Steril* 2002;77:932–935
26. Conway GS, Jacobs HS: Leptin: A hormone of reproduction. *Hum Reprod* 1997;12:633–635
27. Remsberg KE, Talbott EO, Zborowski JV, *et al.*: Evidence for competing effects of body mass, hyperinsulinemia, insulin resistance and androgens on leptin levels among lean, overweight, and obese women with polycystic ovary syndrome. *Fertil Steril* 2002;78:479–486
28. Stein IF, Leventhal ML: Amenorrhea associated with bilateral polycystic ovaries. *Am J Obstet Gynecol* 1935;29:181–187

29. Achard C, Thiers J: Le virilisme pileux et son association à l'insuffisance glycolytique (diabète à femmes de barbe). *Bull Acad Natl Med (Paris)* 1921;86:51–66
30. Poretsky L, Kahin MF: The gonadotropic function of insulin. *Endocrinol Rev* 1987;8:134–141
31. Moller DE, Flier JS: Detection of an alteration in the insulin receptor gene in a patient with insulin resistance, acanthosis nigricans and the polycystic ovary syndrome. *N Eng J Med* 1988;319:1526–1529
32. Walder K, Flippis A, Clark S, *et al.*: Leptin inhibits insulin binding in isolated rat adipocytes. *J Endocrinol* 1997;155:R5–R7
33. Cohen B, Novich D, Rubinstein M: Modulation of insulin activities by leptin. *Science* 1996;274:1185–1188
34. Karlsson C, Lindell K, Svensson E, *et al.*: Expression of functional leptin receptors in the human ovary. *J Clin Endocrinol Metab* 1997;82:4144–4148
35. Spicer JL, Francisco CC: The adipose obese gene product, leptin: Evidence of a direct inhibitory role on ovarian function. *Endocrinology* 1997;138:3374–3379
36. Jacobs HS: Familial aspects of polycystic ovary syndrome. *Gynecol Forum* 1997;2:8–10
37. Dunaif A, Xia J, Book CB, *et al.*: Excessive insulin receptor phosphorylation in cultured fibroblasts and in skeletal muscles. *J Clin Invest* 1995;96:801–810
38. Zhang LH, Rodrigues H, Ohno S, *et al.*: Serine phosphorylation of human p450 c17 increases 17,20 lyase activity: Implications for adrenarche and the polycystic ovary syndrome. *Proc Natl Acad Sci* 1995;92:10619–10623