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Serum Immunoreactive Leptin Concentrations in Women with Polycystic Ovary Syndrome*

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ABSTRACT. Recent data in the mouse demonstrate that leptin, a protein hormone produced by fat cells, is required for fertility. In the absence of leptin the mice become obese, diabetic and infertile. Polycystic ovary syndrome (PCOS), a common cause of infertility in women, is associated with obesity and insulin resistance. Because of the increased frequency of PCOS in obese women we tested the hypothesis that alterations in serum leptin concentrations might be associated with PCOS. Immunoreactive leptin concentrations were measured in 58 women with PCOS and 70 regularly menstruating (control) women. As has previously been shown there was a positive correlation between leptin levels and body mass index (BMI). Although the leptin levels in the majority of women with PCOS fell within the control range, 29% of PCOS women had leptin levels above the 99% prediction interval for their BMI and none had low leptin levels. There were also positive correlations of leptin levels with free testosterone and insulin sensitivity in control women. In women with PCOS, 13% and 9.5% exhibited higher than expected leptin concentrations with respect to free testosterone and insulin sensitivity, respectively. Insulin resistant PCOS women had higher leptin levels than controls. The data demonstrate that a substantial proportion of women with PCOS have leptin levels that are higher than expected for their BMI, free testosterone and insulin sensitivity. These results suggest that abnormalities in leptin signaling to the reproductive system may be involved in certain cases of PCOS.

IT IS WELL known that extremes of body mass are associated with disturbances of reproductive function in women. Women with a low percentage of body fat such as trained distance runners, ballet dancers, and women with anorexia nervosa often are infertile (1, 2). At the other extreme, obese women exhibit a high incidence of oligo- or amenorrhea and infertility (1, 3, 4). In light of these observations it has been proposed that an endocrine signal communicates the nutritional status and the degree of fat stores to the reproductive axis.

Leptin is a recently identified 16,000 MW protein hormone produced by adipocytes (5) that has been shown to increase general metabolism and decrease appetite (6-9). Genetically obese *ob/ob* mice fail to produce functional leptin protein and become extremely obese, develop insulin resistance that progresses to diabetes, and are infertile (10). Injection of leptin into *ob/ob* mice increases the levels of circulating gonadotropins (11), promotes ovarian follicular development (11), and restores fertility (12). The mechanism of the effects of leptin on the reproductive axis remain unknown. The discovery of leptin receptor mRNA in the brain and the ovary (13-15) suggests that leptin may act centrally to alter hypothalamic and/or pituitary function and that leptin may promote ovarian function through direct actions on the ovarian follicle.

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Polycystic ovary syndrome (PCOS) is a heterogeneous syndrome characterized by oligo- or amenorrhea, hyperandrogenism, and the accumulation of multiple small subcapsular cystic follicles in the ovary (3). Women with PCOS seldom ovulate because follicle growth arrests at the small antral stage and large preovulatory follicles rarely develop. PCOS is frequently associated with obesity and insulin resistance (3, 4), symptoms reminiscent of those observed in leptin deficient *ob/ob* mice. Because of these similarities we tested the hypothesis that women with PCOS have altered levels of circulating leptin.

Materials and Methods

Subjects

The study population consisted of 58 women with polycystic ovary syndrome and 70 regularly menstruating control women. Women with PCOS were less than 45 years of age (19 - 44; mean = 27.3), with oligo/amenorrhea dating to puberty and polycystic ovarian morphology confirmed by visual inspection of the ovaries at laparotomy, laparoscopy or by ultrasound examination. There was no evidence of hyperprolactinemia, Cushing's syndrome, congenital or non-classical adrenal hyperplasia, thyroid disease, or hormone secreting tumors. Control women were healthy, less than 45 years of age (19 - 44; mean = 33.6) with regular menstrual cycles and no evidence of hyperandrogenism, polycystic ovaries, endometriosis or abnormal uterine bleeding. Neither PCOS nor control women had taken medications within 60 days of blood collection.

After obtaining informed consent fasting serum samples were obtained and immediately frozen (-80° C) until hormone assays were performed.

Hormone assays

Serum leptin was measured in duplicate with a human leptin radioimmunoassay kit using recombinant human

leptin as standard (Linco Research, Inc., St. Charles, MO). The intra- and inter-assay coefficients of variation were 4.2% and 4.5%, respectively. Insulin was measured with an RIA kit and LH and FSH were measured with IRMA kits (Diagnostic Systems Laboratories, Webster TX). The standard for LH was the WHO 2nd International Standard for Human Pituitary Luteinizing Hormone (80/552) and the standard for FSH was the 2nd International Reference Preparation of Pituitary Follicle Stimulating Hormone (2nd IRP 78/549). Total testosterone, free testosterone and dehydroepiandrosterone sulfate were measured with coated tube immunoassay kits (Diagnostic Products Corporation, Los Angeles, CA).

Determination of insulin sensitivity

In a subset of women (22 with PCOS and 19 controls) in vivo insulin sensitivity was determined using minimal modeling analysis of frequently sampled intravenous glucose tolerance tests as previously described (16, 17). These studies were approved by the UCLA Medical Center Human Subject Protection Committee and were performed in the UCLA Clinical Research Center. All women gave informed consent. The insulin sensitivity index was calculated using the minimal modeling technique (16). This method has been shown to yield results equivalent to the euglycemic glucose clamp technique (16).

Statistical analysis

Differences between groups were determined by Kruskal-Wallis ANOVA on ranks followed by pairwise comparisons using Dunn's test. Correlations were calculated by least squares linear regression. Significance was considered to be $P < 0.05$.

Results

Immunoreactive leptin concentrations were measured in 128 women of reproductive age. The women were classified as lean if their body mass index (BMI) was less than 25 kg/m^2 and obese if their BMI was ≥25 kg/m^2. As shown in Fig. 1, regularly cycling control women had significantly increased leptin levels if they were obese compared to lean ($P < 0.001$). Leptin concentrations in lean women with PCOS were elevated compared to lean controls ($P < 0.005$) and were similar to the concentrations in obese controls. In obese women

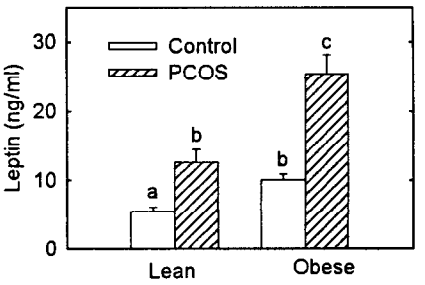

FIG. 1. Serum leptin concentrations in lean and obese women with PCOS. Women were divided into lean (BMI <25 kg/m^2) PCOS (n=29), obese (BMI >25 kg/m^2) PCOS (n=29), lean control (n=30), and obese control (n=40). Serum leptin concentrations were measured by RIA. Groups with different letters are significantly different.

with PCOS the circulating leptin levels were significantly higher than lean women with PCOS and all control women ($P < 0.001$). Although women with PCOS have increased mean leptin levels compared with regularly cycling control women there was substantial overlap in the individual leptin levels of women with PCOS and the controls. When serum leptin levels were analyzed as a function of BMI (Fig. 2A) an interesting pattern emerged. As has previously been shown (18, 19), linear regression analysis revealed a positive correlation of leptin with BMI for the control women ($r = 0.459$; $P < 0.001$). Although the leptin levels of the majority of PCOS women and all of the control women fell within the 99% prediction intervals for the control population, 17 (29%) of the PCOS women had leptin levels that were above the 99% prediction interval. Surprisingly the BMIs of these women spanned the range of BMIs for the subject population and were not limited to obese women. There were no instances of low leptin levels in PCOS women. When leptin levels were analyzed with respect to waist:hip ratio there was a positive correlation in the control women ($r = 0.431$; $P < 0.001$) but no significant correlation in PCOS women ($r = 0.235$; $P = 0.281$). These data support the conclusion that a substantial proportion of women with PCOS have leptin levels that are significantly higher than would be

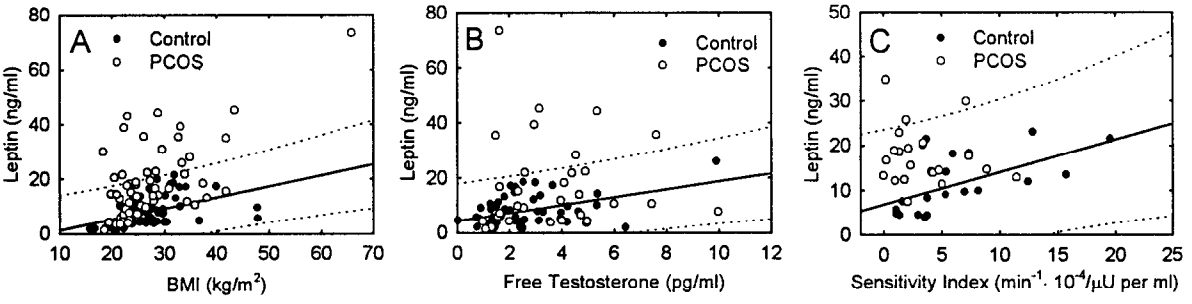

FIG. 2. Correlations between serum leptin concentrations and body mass index, free testosterone, and insulin sensitivity. The solid lines represent the linear correlation for the control women. The dotted lines are the 99% prediction intervals for the control population. A. The linear correlation (solid line) between leptin and BMI for control women ($r = 0.459$; $P < 0.001$). The dashed line is the linear regression line between leptin and BMI for PCOS women ($r = 0.638$; $P < 0.001$). B. The linear correlation between leptin and free testosterone for control women ($r = 0.449$; $P < 0.001$). C. The linear correlation between leptin and insulin sensitivity index for control women ($r = 0.576$; $P < 0.01$).

expected for a given body mass in regularly cycling women.

A high proportion of women with PCOS are hyperandrogenic. As expected, free testosterone levels in the PCOS women (3.7 ± 0.4 pg/ml) were increased (P = 0.008) compared to regularly cycling control women (2.5 ± 0.2 pg/ml). As shown in Fig. 2B, control women exhibited a positive correlation between leptin and free testosterone concentrations (r = 0.449; P <0.001). As with BMI there was a subset (13%) of the women with PCOS that had leptin levels higher than the 99% prediction interval for the control population. As a consequence there was no significant correlation between free testosterone and leptin levels (r = 0.009; P = 0.96) in women with PCOS as a whole. All of the women with high leptin levels were included in the subset of PCOS women that exhibited high leptin levels with respect to BMI. There was a virtually identical positive correlation between leptin levels and total testosterone in control women (r = 0.470; P < 0.001) but no significant correlation in women with PCOS (r = 0.083; P = 0.954).

Insulin resistance is frequently associated with PCOS and with obesity. To determine if there was an association of leptin levels with insulin resistance, leptin concentrations were measured in women whose insulin sensitivity was characterized by frequently sampled intravenous glucose tolerance tests. The insulin sensitivity index which is a measure of the increase in the fraction of the extracellular glucose pool that disappears per minute as a result of a unitary increase in serum insulin concentration was calculated by minimal modeling analysis (16). Insulin resistant women have a low sensitivity index. As shown in Fig. 2C, there was a positive correlation of serum leptin levels with the insulin sensitivity index in control women (r = 0.576; P <0.01). The majority of women with PCOS had leptin levels that fell within the 99% prediction intervals for the control population but two of the PCOS women (9.5%) had leptin levels slightly above the 99% prediction interval. Both of these women were PCOS women with high leptin levels relative to BMI. Insulin resistant (sensitivity index $<5 \times 10^{-4} \cdot \text{min}^{-1}/\mu\text{U}$ per ml) PCOS women had mean serum leptin levels (18.1 ± 1.6 ng/ml; n = 16) 2-fold higher (P <0.001) than controls (8.5 ± 2.1 ng/ml; n = 10). There was no correlation of leptin with insulin sensitivity in women with PCOS (r = 0.136; P = 0.547) due to significantly higher leptin levels in insulin resistant PCOS women. In control women there were negative correlations between leptin levels and fasting insulin levels (r = 0.448; P < 0.05) and post glucose insulin area under the curve (r = 0.491; P < 0.05) but not in the PCOS women (r = 0.130, P = 0.548 and r = 0.154; P = 0.494, respectively).

Of the 17 women with PCOS that appear to have high leptin levels relative to BMI, there were no hormonal differences compared to PCOS women with leptin levels in the control range (Table 1). Although fasting insulin and free testosterone were higher than control women (P < 0.01) there were no differences relative to other PCOS women. In addition, there were no significant differences in LH, FSH, LH:FSH ratio, total testosterone or dehydroepiandrosterone sulfate compared to controls or PCOS. There was no correlation of leptin levels with age (r = 0.001; P = 0.991) demonstrating that although the PCOS women were somewhat younger than controls, the results were unaffected by the age difference. Thus, it is unclear why this subset of women with PCOS have higher leptin levels than expected for their body mass.

Table 1. Fasting serum hormone concentrations.

	Control	PCOS	
		"normal" leptin	"high" leptin
Leptin (ng/ml)	8.4 ± 0.7	11.4 ± 1.0[a]	34.4 ± 3.2[a,b]
Insulin (μIU/ml)	4.3 ± 0.5	11.1 ± 1.0	19.5 ± 10.1[c]
LH (mIU/ml)	12.3 ± 2.0	14.5 ± 2.9	14.5 ± 2.9
FSH (mIU/ml)	3.5 ± 0.4	3.4 ± 0.4	3.4 ± 0.5
LH:FSH ratio	3.7 ± 0.5	4.0 ± 0.5	4.4 ± 0.9
Free T (pg/ml)	2.4 ± 0.2	3.6 ± 0.4	5.3 ± 1.0[c]
Total T (ng/dl)	55.6 ± 3.6	73.2 ± 7.8	69.3 ± 12.7
DHEAS (μg/dl)	249 ± 24	291 ± 29	265 ± 42

T: testosterone; DHEAS: dehydroepiandrosterone sulfate.
[a]P <0.001 vs control; [b]P <0.001 vs "normal" leptin
[c]P <0.01 vs control

Discussion

The results of this study confirm previously published data demonstrating a positive correlation between serum leptin levels and BMI (18, 19). The relationship between BMI and leptin concentrations was present in women with PCOS as well, but almost one third of women with PCOS had elevated leptin levels compared to regularly cycling controls. This finding suggests that leptin secretion by the adipocytes in some women with PCOS may be abnormally regulated. It appears that their adipocytes may secrete more leptin than controls or alternatively, the metabolic clearance of leptin may be reduced as BMI increases. The only published data regarding effects of leptin on the reproductive system were obtained in mice genetically deficient in bioactive leptin (11, 12). In these mice recombinant leptin treatment restored their fertility demonstrating that low leptin levels cause a reversible form of infertility. The present results indicate that high levels of leptin are also associated with infertility, but the mechanisms by which elevated leptin could interfere with reproductive function remain unknown.

Common findings in PCOS are hyperandrogenism due to increased ovarian androgen production (4) and insulin resistance (20). One hypothesis is that increased insulin levels in women with insulin resistance stimulate the type I IGF receptor on the theca cells, thereby increasing thecal androgen production in response to LH (21). As expected the population of PCOS women studied had elevated free testosterone and fasting insulin levels. In control women there was a positive correlation between leptin and free testosterone suggesting that the increased leptin in PCOS women might be related to the increased free testosterone levels. The data did not support this hypothesis because several PCOS women with relatively low free testosterone levels had very high leptin levels.

Insulin has been shown to increase leptin mRNA in adipocytes, suggesting that insulin may stimulate leptin

secretion (22-24). In control women there was a positive correlation between serum leptin levels and insulin sensitivity. This observation is consistent with the concept that insulin regulates leptin secretion and suggests that adipocytes with greater insulin sensitivity secrete more leptin. The insulin resistant PCOS women had significantly higher leptin levels than insulin resistant controls of similar BMI. This raises the possibility that PCOS women may be more responsive to insulin with respect to leptin secretion than glucose regulation. The concept that certain PCOS women may be insulin resistant with respect to glucose metabolism but that other insulin responses such as androgen production and leptin secretion may be relatively normal is an intriguing hypothesis that should be tested.

These data provide evidence that leptin is a signal to the reproductive axis not only in rodents but also in humans. Data from studies with leptin deficient *ob/ob* mice demonstrated that low leptin levels interfere with reproductive function (11, 12). The present data extend this concept to the other extreme, namely that excessive leptin levels are also associated with disrupted fertility. It remains unclear how elevated leptin levels in PCOS could interfere with reproduction. Leptin receptor mRNA has been found in the brain (13-15) and at high levels in the ovary (13). In *ob/ob* mice leptin treatment increased circulating gonadotropin levels (11) suggesting that leptin may directly affect the hypothalamus and/or pituitary. In this study high leptin levels were not associated with alterations in circulating LH or FSH levels or changes in LH:FSH ratio. These data do not support the concept that increased leptin interferes with reproductive activity through central effects on gonadotropin secretion, however a definitive answer to the question will require pulse analysis studies.

Despite the presence of leptin receptor mRNA in the ovary there are no data demonstrating direct effects of leptin on ovarian cells. Nevertheless, leptin appears to promote ovarian function. It is possible that the PCOS women with high leptin levels may produce a less potent form of leptin protein or they may have a diminished response to leptin at the target cell level. This could be caused by a mutant receptor or a defect in intracellular signaling. Receptor defects have been found in *diabetic* (C57BL/Ks *db/db*) mice and Zucker *fatty* (*fa/fa*) rats (14, 25, 26). Thus, high circulating levels of immunoreactive leptin may be a compensatory response to decreased leptin bioactivity and/or signaling. This is an attractive hypothesis because anovulation in certain women with PCOS could be caused by a deficiency of leptin bioactivity analogous to that found in *ob/ob* mice. Whether a defect in the leptin system exists in PCOS women with increased serum leptin levels and whether such a defect alone is sufficient to cause PCOS or is only a contributing factor will require further study.

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