

## Serum leptin and body composition in polycystic ovarian syndrome

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**Background:** The role of leptin in polycystic ovarian syndrome (PCOS) is unclear. We investigated the relationship between serum leptin levels, body composition and insulin resistance in polycystic ovarian syndrome (PCOS).

**Methods:** We analyzed differences between 27 patients with PCOS and 25 control subjects in serum glucose and leptin levels, insulin resistance, body fat mass, lean body mass, and water volume.

**Results:** Serum leptin was significantly correlated with basal insulin levels, BMI and IR in both groups ( $P < 0.01$ ). Fat mass, fat percentage, lean mass and water volumes were positively correlated and lean percentage and water percentage were negatively correlated with leptin levels ( $P < 0.05$ ). Leptin levels were significantly different between the groups in a multivariate regression analysis after correcting for the difference in BMI and body fat percentage ( $P < 0.05$ ). When the effects of fat percentage on serum leptin were eliminated, the levels were significantly different between the PCOS and control groups, and were statistically more powerful than BMI ( $P < 0.01$ ).

**Conclusion:** These findings support the idea that factors other than excess fat mass or fat-free mass might be important in the regulation of serum leptin levels in PCOS.

**Key words:** Leptin, polycystic ovarian syndrome, body fat, body mass index, insulin resistance

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Leptin, predominantly produced by adipocytes, is a polypeptide hormone essential in the regulation of normal body weight.<sup>1</sup> The production of leptin is under neuroendocrine control and leptin receptors are found in various endocrine organs, including the pancreas and ovaries.<sup>2</sup> However, the role of leptin in reproductive function as well as the interaction between reproduction and nutrition are not yet clear.<sup>2-8</sup> The role of leptin in polycystic ovarian syndrome (PCOS) is under investigation since the disease involves impairment of reproduction and nutrition. Recent studies have reported the interesting finding that insulin sensitizers could influence the pathophysiology of PCOS, decreasing serum leptin as well as insulin levels and resulting in improvements in reproductive functions.<sup>2,7</sup> We investigated circulating leptin levels and their relationship with the amount and percentage of body fat as well as insulin and glucose levels during a standard 75-gram oral glucose tolerance test (OGTT) in infertile patients with PCOS.

### Methods

Twenty-seven women with the diagnosis of PCOS attending our infertility clinic made up the study group. The diagnosis of PCOS was based on 1) oligomenorrhea or amenorrhea or 2) anovulation ( $< 3$  ng/mL mid-luteal

progesterone levels) and 3) ultrasound evidence of bilateral enlarged polycystic ovaries. Ovulatory partners of 25 infertile couples were included as control subjects. Informed consent was obtained from patients and controls for participation in the study.

A 2-hour 75-gram OGTT was performed for each case after overnight fasting, following a 3-day standardized high carbohydrate diet. Insulin and glucose levels were measured initially and at 60 and 120 minutes. Initial blood samples for OGTT were obtained between 8:30 AM and 10:00 AM. Insulin resistance (IR) was calculated with the formula for Homeostasis Model Assessment (HOMA IR = fasting serum insulin ( $\mu$ U/mL)  $\times$  fasting plasma glucose [mmol/L]/22.5). Body mass index (BMI) was calculated for each case as weight (in kilograms) divided by height (in meters) squared. Each case underwent bioelectric impedance (BEI) analysis to estimate body composition. Resistance and reactance were measured with the subject lying supine by use of an impedance analyzer with two body surface electrodes and a conduction current less than 1  $\mu$ A and 50 kHz (Bodystat 1500, Body Composition Analyzer). One surface electrode was placed on the dorsal surface of the right hand and one on the dorsal surface of the right foot. Body fat and lean body mass (fat-free mass) and their percentage values were automatically calculated by the analyzer. Water volume and percentage of water were also obtained with the same analyzer.

Hormonal and biochemical determinations were performed at the Central Laboratory of the Medical Faculty, Ondokuz Mayıs University. Insulin levels were measured using the Immulite 2000 immunometric method. Leptin measurements were obtained with the DSL-10-23100 Human Leptin ELISA kit by ELISA technique. Hormonal evaluations as well as the BEI analyses were made in the early follicular phase in all the control cases and if possible in the PCOS cases; otherwise they were made randomly in severe oligomenorrheic or amenorrheic women.

Data were analyzed with the SPSS statistical package program. The Mann-Whitney test was used for comparing groups and Pearson correlation analysis to test for correlations. Multivariate regression analyses were performed to evaluate the effects of certain parameters on serum leptin levels in PCOS. A  $P$  value  $<0.05$  was considered statistically significant.

### Results

The differences in leptin levels, basal insulin levels, IR and BMI between the PCOS and control groups were statistically significant (Table 1). Body fat mass, lean body mass, water content and the percent values were also significantly different. There was no correlation between leptin levels and age, except for the control group ( $P<0.05$ ) (Table 2). Leptin levels correlated with basal insulin levels, BMI and IR in the PCOS and control groups and overall (Table 2).

In the multivariate analysis, leptin levels were significantly increased in the PCOS group compared with the control group even when the effects of BMI and body fat mass were eliminated. The effect of BMI on leptin levels appeared to be considerably stronger than the effect of the body fat mass ( $P<0.01$  and  $P<0.05$ , respectively). The difference between groups for IR was significant when the effect of body fat was eliminated, but this was not the case when the effect of BMI was disregarded.

Body fat mass, fat percentage, lean body mass and water volumes were positively correlated and lean percentage and water percentage were negatively correlated with leptin levels in all cases when study and control groups were considered together ( $P<0.05$ ) (Table 2). When the PCOS and control groups were analyzed separately, all the previously significant correlations disappeared in the PCOS group, but persisted in the control group, except for water percentage (Table 2). There was a negative correlation between impedance and leptin levels, which was again significant in the control group, but not in the PCOS group.

### Discussion

Leptin, a hormonal product of the *ob* (obese) gene, is expressed by adipocytes and is thought to play a role in the regulation of food intake and metabolism. Leptin might

serve as a permissive signal for the reproductive system.<sup>4</sup> Leptin levels are not constant and may vary with follicular growth and maturation and thus fluctuate throughout the menstrual cycle.<sup>9</sup> Additionally, nocturnal changes in luteinizing hormone pulse parameters are associated temporarily with the rise of plasma concentrations of leptin at night, and the fluctuations of plasma leptin concentrations are synchronous with those of LH and estradiol.<sup>10</sup> In our study, we investigated basal leptin levels in the morning and in the early follicular phase of the menstrual cycle, except in patients with steady-state hormonal status.

The consensus is that leptin levels are high in PCOS patients,<sup>7,11,12</sup> but the data on the relationship of leptin and fat distribution in PCOS syndrome are conflicting.<sup>13</sup> In our study, we observed that serum leptin levels as well as basal insulin levels, IR and BMI were significantly high in PCOS patients. This is in accordance with reports in the literature that show that leptin is strongly correlated with MI.<sup>5,6,11,12,14</sup> Although leptin production mainly occurs in adipose tissue, when the difference in body fat mass between PCOS and controls was corrected for, the difference in the leptin levels remained significant. This finding makes us think that there might be other reasons for the increase in the serum leptin concentration in PCOS cases. Our observation was in contrast with a previous article, which reported that serum leptin concentrations were almost exclusively determined by the amount of body fat.<sup>6</sup> On the other hand, there are also data in the literature on the relationship between leptin and fertility that support our observations: Serum leptin levels decreased significantly after 2 months of metformin treatment in obese PCOS patients.<sup>15</sup> Those authors suggested that the decrease in leptin could not, however, be explained by the changes in body weight, because the BMI of their patients remained constant during therapy.<sup>15</sup>

Imani et al showed that the prediction of which patients would be anovulatory after clomiphene citrate medication could be slightly improved (area under curve increased from 0.82 to 0.85) by using leptin instead of BMI or the waist-to-hip ratio.<sup>16</sup> This may indicate that leptin is more directly involved with the ovarian function or dysfunction in these patients. According to Fedorcsak, obesity directly affects ovarian function in PCOS at least in part by increased intra-follicular leptin levels, and may induce a relative resistance to gonadotropin stimulation.<sup>17</sup> However, the discussion is not over yet.<sup>18</sup> We found that leptin levels were higher along with body fat mass and fat percentage in the PCOS group compared to the control group, as would be expected since plasma leptin levels tend to increase in parallel with adipose stores and are strongly correlated with body fat mass.<sup>19</sup> However, others argue that the large variation in circulating leptin concentrations at similar levels of adiposity indicates that factors other than fat mass may be important in regulating serum leptin.<sup>19</sup> This argument was supported by

**Table 1.** Age, body mass index, serum leptin levels, oral glucose tolerance test, insulin resistance and body fat composition values in the polycystic ovarian syndrome and control groups.

	PCOS (n=27) Mean±SD	Control (n=25) Mean±SD	P value
Age (years)	28.56 ± 4.66	29.76 ± 6.62	NS
BMI (kg/m <sup>2</sup> )	29.19 ± 5.51	23.96 ± 2.85	<0.01
Leptin (ng/mL)	28.48 ± 12.90	14.84 ± 7.16	<0.01
Insulin basal (μU/mL)	17.39 ± 8.06	9.14 ± 3.42	<0.01
Insulin 1h (μU/mL)	64.03 ± 42.17	41.40 ± 28.23	<0.05
Insulin 2h (μU/mL)	50.33 ± 55.56	26.11 ± 25.48	NS
Glucose basal (mg/dL)	93.89 ± 17.54	92.04 ± 10.95	NS
Glucose 1h (mg/dL)	135.78 ± 42.53	118.60 ± 33.15	NS
Glucose 2h (mg/dL)	115.30 ± 31.03	100.12 ± 22.65	NS
Insulin Resistance (IR)	4.07 ± 2.09	2.13 ± 0.98	<0.01
Body fat mass (kg)	29.63 ± 13.23	20.84 ± 5.11	<0.01
Fat %	36.88 ± 7.56	31.78 ± 4.73	<0.05
Lean body mass (kg)	47.49 ± 6.29	43.79 ± 4.69	<0.05
Lean %	61.85 ± 8.38	68.30 ± 4.70	<0.01
Water content (L)	34.30 ± 5.01	31.52 ± 2.93	<0.05
Water %	44.49 ± 5.41	48.95 ± 3.69	<0.01
Impedance (Ohms)	568.46 ± 76.83	602.42 ± 74.08	NS

IR = fasting serum insulin (μU/ml) x fasting plasma glucose (mmol/liter)/22.5; NS=non-significant

**Table 2.** Correlations between leptin and age, basal insulin levels, body mass index, insulin resistance, body fat composition and impedance in the overall, control and PCOS groups.

	Overall (n=52)		Control group (n=25)		PCOS group (n=27)	
	Correlation coefficient	P value	Correlation coefficient	P value	Correlation coefficient	P value
Age (years)	0.092	NS	0.397	<0.05	0.060	NS
Insulin basal (μU/ml)	0.795	<0.01	0.755	<0.01	0.698	<0.01
BMI (kg/m <sup>2</sup> )	0.727	<0.01	0.783	<0.01	0.577	<0.01
Insulin Resistance (IR)	0.741	<0.01	0.730	<0.01	0.620	<0.01
Fat %	0.495	<0.01	0.554	<0.01	0.313	NS
Fat (kg)	0.502	<0.01	0.708	<0.01	0.304	NS
Lean %	-0.440	<0.01	-0.580	<0.01	-0.179	NS
Lean (kg)	0.415	<0.01	0.490	<0.05	0.233	NS
Water %	-0.442	<0.01	-0.207	NS	-0.290	NS
Water (L)	0.416	<0.01	0.528	<0.01	0.232	NS
Impedance (Ohms)	-0.308	0.05	-0.509	0.05	-0.090	NS

IR=fasting serum insulin (μU/ml) x fasting plasma glucose (mmol/liter)/22.5; NS=non-significant

our study as well, with the observation that the body fat mass-leptin correlation in our PCOS group disappeared.

We also agree with Laughlin et al, who speculated that the opposing effects of hyperinsulinemia (stimulatory) and adipocyte IR (negative) specific to PCOS might negate the impact of insulin excess and account for the maintenance of normal levels of serum leptin in PCOS.<sup>7</sup> Body fat mass and fat percentages were positively correlated with leptin levels and, as expected, lean body mass percentage was negatively correlated. Interestingly, lean body mass (fat-free mass) was positively correlated with leptin levels. All of these relationships were absent in the PCOS group, a finding consistent with the report that leptin positively correlated with the fat-free mass in men ( $P < 0.01$ ) but not in women<sup>20</sup> ( $P > 0.05$ ).

Jinno et al investigated bioelectrical impedance (BEI) as a novel method for assessing assisted female fertility.<sup>21</sup> In the stepwise logistic regression analysis of five factors (BEI on luteal day 4 prior to the IVF cycle [BEI-L4], age, basal FSH, body height and BMI), BEI-L4 alone appeared to be a significant predictor ( $P < 0.05$ ) of achievement of pregnancy

by IVF.<sup>21</sup> In our study, we did not find a significant difference between the early follicular phase BEI values of PCOS and control subjects. Leptin and BEI findings were correlated in the overall and the control group, but disappeared in the PCOS patients, as did the other fat distribution parameters.

High serum leptin, insulin and testosterone levels together with reduced insulin sensitivity have been observed in obese PCOS women, suggesting that high leptin levels could be characteristic of the obese PCOS phenotype.<sup>15</sup> In this study, we also found that the degree of IR was higher in PCOS despite the adjustment of the body fat mass between the two groups.

In summary, we found positive correlations between leptin and body fat mass, fat percentage and a negative correlation with fat-free mass percentage. Interestingly, there was also a positive correlation with fat-free mass. None of these correlations could be demonstrated in the PCOS patients alone, suggesting that factors other than excess fat and excess fat-free mass may be important in the regulation of serum leptin, and consequently, female reproductive function.

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