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# Relationships between free leptin and insulin resistance in women with polycystic ovary syndrome

***Javad Mohiti-Ardekani[1] Ph.D., Nasim Tarof[1] M.Sc., Abbas Aflatonian[2] M.D.***

1 Department of Biochemistry and Molecular Biology, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
2 Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

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## Abstract

**Background:** Leptin is an adipokine that circulates in a free form and bound to a soluble leptin receptor**.** Patients with polycystic ovary syndrome have increased insulin resistance and high incidence of obesity.

**Objective:** This study was carried out to evaluate levels of leptin and free leptin in women with polycystic ovarian syndrome (PCOS) and note any relationships with insulin resistance and adiposity.

**Materials and Methods:** We assessed the correlation of metabolic parameters with the levels of free leptin and it's bound form in 27 PCOS women (aged 26±5.6 years) and 27 healthy women with normal menstrual cycle as controls (aged 25 ±4 years).Total leptin and insulin levels were measured using ELISA. Free leptin form was purified by Gel filtration chromatography and their collected fractions were measured by a sensitive ELISA-Kit. Insulin resistance was calculated by homeostasis model assessment (HOMA).

**Results:** In PCOS patients and control group a correlation between leptin and body mass index (BMI) was found. A significant difference was found between leptin and free leptin levels in PCOS subjects and controls ($p<0.05$). Significant correlations were found between free and total leptin with insulin resistant in PCOS subjects ($r=0.78$ $p=0.00$, $r=0.84$ $p=0.003$) and control groups respectively ($r=0.86$ $p=0.00$, $r=0.69$ $p=0.00$).

**Conclusion:** Total and free leptin forms are correlated significantly with BMI in patients with PCOS and in controls. Total and free leptin forms showed significant correlations with insulin resistance but no significant difference was seen in the two groups investigated**.**

***Key words****: Free leptin, Polycystic Ovarian Syndrome (PCOS), Insulin resistance.*

## Introduction

Leptin, the gene product of the ob gene, is thought to provide the central nervous system with feed-back information about fat storage of the body (1). Thus, leptin is thought to be a part of the regulation of appetite, food intake and the lipid metabolism (2).

**Corresponding Author**:
*Javad Mohiti-Ardekani*, Department of Biochemistry and Molecular Biology, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
**E-mail:** mohiti_99@yahoo.com

Obese humans present with hyperleptinemia as an indicator of leptin resistance which plays a major role in the pathogenesis of obesity (3). Polycystic ovary syndrome (PCOS) is among the most common endocrine disorders, affecting more than 5% of women of reproductive age (4, 5). An association of PCOS with peripheral insulin resistance, compensatory hyperinsulinemia and alterations in ß-cell function as the cause of its predisposition to develop a metabolic syndrome (type 2 diabetes mellitus, hypertension, lipid disorders, obesity) has been established (6). According to the majority of studies, most PCOS

women are insulin resistant and overweight or obese (7-11). In mice, a genetic defect in the ob gene results in severe obesity and type 2 diabetes mellitus, as well as in infertility (6).

In this mouse model, leptin injections restore fertility. Leptin treatment in normal female mice accelerates puberty (12). In humans, granulosa cells have been shown to secrete leptin (13) and leptin seems to influence adrenal androgen formation (14).

However, the role of leptin in ovarian function or on the reproductive system remains unclear. It was shown that leptin levels did not influence follicular maturation and ovulation in PCOS patients (10) while Carmina *et al* demonstrated a negative correlation between leptin and dehydroepiandrosterone sulphate (DHEAS) levels in 21 lean PCOS women, suggesting that androgens may play a role in suppressing serum leptin (15).

Another study showed that leptin concentrations correlated inversely with luteinizing hormone (LH) levels, independent of body weight and insulin resistance (16). In contrast again, Laughlin *et al* (17) proposed that leptin is neither involved in the hypersecretion of LH nor in the regulation of LH pulsatility. During lactational amenorrhea Sir-Petermann *et al* (18) also showed that leptin secretion is not related to LH secretion. Plasma leptin is in free and bound forms in circulation (19).

The free leptin has been shown to correlate with total serum leptin levels and BMI in women of reproductive age. Kado *et al* (20) hypothesized that obese women are able to conversely increase their leptin activity by minimizing the bound leptin fraction, thus influencing the biological activity of leptin. In line with this, Sinha *et al* (21) reported that in lean subjects, leptin circulates predominantly in the bound form, whereas in obese patients, leptin circulates mostly in the free form. Similar results were found for Japanese men and women, showing a direct correlation between free leptin level and BMI and parameters of insulin resistance (22).

Weight loss, following gastric restrictive surgery, leads to a reduction in circulating leptin levels and to a decrease free leptin (23). In adolescent girls with anorexia nervosa, free leptin and Free Leptin Index (FLI) but not total leptin levels were closely related to parameters of sexual maturation and the onset of puberty (26). Therefore, to elucidate the free leptin role in PCOS, we purified the free leptin in PCOS patients and healthy control and analyzed the interrelations between serum total leptin and free forms with insulin and insulin resistance and the other metabolic factors**.**

## Materials and methods

### Study population

The study was carried out on 54 women (27 PCOS subjects and 27 voluntary BMI, and age matched healthy women with normal menstrual cycle as controls). Clinical diagnosis of PCOS was made from the 1990 National Institutes of Health (NIH) conference, when either oligomenorrhea (cycles lasting longer than 35 days) or amenorrhea (less than two menstrual cycles in the past 6 months) and either clinical signs of hyperandrogenism (hirsutism or obvious acne or alopecia and/or an elevated total testosterone) were found, and other pituitary, adrenal or ovarian diseases could be excluded. PCOS as well as control subjects had not taken any medication known to affect carbohydrate metabolism or endocrine parameters for at least 3 months before entering the study. In controls, LH, FSH and insulin levels were estimated in the early follicular phase (second or third day of menstruation) from fasting blood samples.

### Data collection

In PCOS subjects and control women, clinical parameters were assessed by physical examination, and BMI was calculated as (weight/height$^2$) (kg/m$^2$**).**

Insulin resistance was calculated by homeostasis assessment model (HOMA) using the formula HOMA-IR = Fasting insulin (μU/ml) × Fasting glucose (mg/dl)/22.5 (27). Low-IR values indicate high insulin sensitivity, whereas high-HOMA values indicate low insulin sensitivity (insulin resistance).

### Biochemical assays

Leptin were measured using ELISA kits [Diagnostic Systems Laboratories (DSL), Webster, TX, USA]. The DSL ACTIVE Human Leptin ELISA is an enzymatically amplified 'two-step' sandwich-type immunoassay. The theoretical sensitivity or minimum detection limit as calculated by interpolation of the mean plus two standard deviations of 12 replicants of the zero standard was 0.05 ng/ml. Intra-assay variation was <5% and interassay variation was <8% for all measured parameters. Insulin concentrations were

measured by sandwich ELISA (Webster, Texas 77598-4217 USA, DSL).

Free leptin form was purified by Gel filtration methods as bellow (20). This study was approved by the local ethical committee and each patient gave written informed consent.

**Purification of free leptin form in serum**

To obtain a standard curve, one vial containing 1 mg lyophilized leptin (Sigma) was dissolved in 0.5 ml HCL (1.5mM) and neutralized with 0.25 ml NaOH (7.5 mM) and applied with Marker Gel filtration Bludextran (Product Brand: Sigma Product Number: D4772) to Sephadex G-100 column chromatography (Amersham Biosource (1908-7101) (9/30 column).

The leptin was eluted with 0.25 mM phosphate buffer (Ph=7.4). Fractions eluting were collected and assayed by a Sensitive ELISA Kit (Catalogue Number: kap 2281: 96 determinations. Manufactured by: Biosource Europe S-A). It showed a single bound indicating free leptin fraction.

Serum sample (0.5ml) was fractionated by Sephadex G-100 gel filtration chromatogheraphy. Fractions eluting between void and bed volumes were assayed by the ELISA kit.

**Statistical analysis**

Data are presented as median plus range for non-parametric data. For better comparison, means ±S.D. is also shown. Correlations between variables were examined by Spearman's correlation coefficient ($r_s$) because analyzed data were not normally distributed. Differences between the groups were evaluated with the Mann–Whitney U test. p values <0.05 were considered significant.

## Results

Patients with PCOS showed significantly higher concentrations of leptin (mean 21±9 ng/ml) *vs.* control (16±10 ng/ml, p=0.042), free leptin (mean 7.3±1.2) *vs.* control (mean 4.3±1.3 ng/ml, p=0.011) and LH (mean 76±18 IU/ml) *vs.* control (mean 2.87±1.93, p=0.001) in plasma (Table I).

We observed no marked difference in insulin level between patients with PCOS (mean 26±13µIU/ml) *vs.* control (mean 25±13) and FSH level between patients with PCOS (8.69±15 IU/ml) *vs.* control (mean 6.56±1.46) (Table I). Furthermore, we analyzed the relationships between leptin, free leptin with BMI, insulin, insulin resistance and LH in PCOS and control groups.

Significant correlations were observed between free leptin and BMI ($r_s$=0.78, p=0.000), insulin resistance (IR) (r=0.53, p=0.004) and insulin (r=0.93, p=0.000) in PCOS patients. Significant correlations were also observed between free leptin and BMI (r=0.86, p=0.00), IR (r=0.57, p=0.003) and insulin (r=0.91, p=0.000) in control group (Table II). No significant correlation was observed between total and free leptin with LH.

**Table I.** Characteristic of control (n=27) and PCOS patients (n=27). Values are means ±SD (median and range).

| Variable | Controls | PCOS | p-value |
|---|---|---|---|
| Age(years) | 25±4 | 26±5.6 | 0.56 |
| BMI | 27.7±2 | 31±6 | 0.34 |
| Leptin(ng/ml) | 16±10 | 21±9 | 0.042 |
| free leptin(ng/ml) | 4.3±1.3 | 7.3±1.2 | 0.011 |
| FBS(mg/dl) | 93±8 | 79±11 | 0.016 |
| Insulin(µIU/ml) | 25.84±13 | 26.88±13 | 0.78 |
| LH(Iu/ml) | 2.87±1.93 | 76±18 | 0.001 |
| FSH(Iu/ml) | 6.56±1.46 | 8.69±15 | 0.47 |

**Table II.** Correlation of leptin and free leptin levels with metabolic parameters in PCOS and control women.

| Variable | Control group | | | | PCOS patients | | | |
|---|---|---|---|---|---|---|---|---|
| | Leptin (ng/ml) | | Free leptin | | Leptin | | Free leptin | |
| | r | p | r | p | r | p | r | p |
| BMI | 0.69 | 0.000 | 0.86 | 0.00 | 0.84 | 0.039 | 0.78 | 0.00 |
| Insulin | 0.61 | 0.001 | 0.91 | 0.00 | 0.69 | 0.05 | 0.93 | 0.00 |
| IR | 0.71 | 0.002 | 0.57 | 0.003 | 0.69 | 0.00 | 0.53 | 0.004 |
| FBS | 0.77 | 0.5 | 0.34 | 0.75 | 0.34 | 0.8 | 0.16 | 0.41 |
| LH | -0.039 | 0.051 | -0.032 | 0.009 | -0.024 | 0.022 | -0.0039 | 0.0084 |

## Discussion

Leptin levels are increased in obesity and may play a role in the development of insulin resistance (7). Our study showed a significant high total and free leptin in PCOS patients as it is compared with controls (Table I) ($p<0.05$). Total leptin levels correlated significantly with BMI in both PCOS women and controls. Leptin correlated with other metabolic parameters including insulin resistance and insulin level in both groups. Similar results were found in PCOS patients from Australia (29), Brazil (16), Canada (10), Finland (30), Italy (15), Sweden (8), Turkey (11) and USA (17, 31). Significant correlation of total leptin with HOMA IR reflected a degree of insulin resistance in both controls and test groups (Table II). We can place our results along the large group of studies, which declare an important role of leptin in pathogenesis of insulin resistance (1, 2, 4, 6, 8, 11, 15, 20, 28, 29**).**

In addition, we found free leptin levels in PCOS patient and control group correlate with parameters of insulin, and insulin resistance (Table II).

Thus free leptin appears to be the appropriate form of leptin which it contributes to insulin resistance and leptin resistance. Potential mechanisms that may mediate leptin resistance include impairment of brain leptin transport or leptin receptor internalization, and receptor post-receptor signaling defects. Furthermore, the active hormone may be reduced by binding proteins or soluble receptors (32). Soluble leptin receptor represents the main leptin-binding compound in plasma, thus regulating its free fraction in the circulation (33).

A considerable portion of circulating leptin is free form which is affected by the degree of adiposity and nutritional state (35-37). We found a positive correlation between free leptin with BMI in PCOS patients and controls. Yannakoulia and co-workers (24) demonstrated that Free leptin index is correlated with total energy intake and inversely correlated with energy intake from dietary fat. They speculated that the macronutrient composition of the diet influences serum concentrations of free leptin. The authors hypothesized that the increase of free leptin levels represents a compensatory mechanism to overcome leptin resistance.

A recently published twin study of pubertal females studying the genetic and environmental influences on the variations of leptin and free form levels showed that leptin is mostly influenced by body composition **(**25, 34). We have demonstrated the correlation of free leptin with insulin resistance in diabetes patients (38). In conclusion, a considerable part of plasma leptin is in free form in circulation.

Although the physiological function of bound and free leptin are not well understood, it has been hypothesized that leptin is more active in its free form because this form is present in cerebrospinal fluid (CSF) (34). We found a relationship between total leptin levels, and free leptin with BMI, insulin resistance, and insulin levels in overweight PCOS patients and their matched control group.

However, further studies are needed to investigate the effect of factors including food or body composition, and genetic on high level of free leptin in PCOS patients.

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