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Serum leptin level in obese women with polycystic ovary syndrome, and its relation to insulin resistance

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ABSTRACT

Objective: To compare serum leptin levels in obese women with polycystic ovary syndrome (PCOS) and normal ovulatory obese subjects in Saudi Arabia, and to evaluate the interrelationship between leptin concentration, sex hormones, and insulin resistance. **Methods:** The present study was conducted on 40 women with PCOS (34.30 ± 2.08 years, body mass index (BMI) 34.84 ± 4.77 kg/m^2, mean ± SD) and 16 obese women as control group (28.10 ± 4.61 years, BMI 33.59 ± 1.23 kg/m^2). Diagnostic criteria for PCOS based on the presence of two out of three traits including oligo –and/or anovulation, clinical and/or biochemical signs of hyperandrogenism and the presence of polycystic ovaries on ultrasound scan. Concentrations of testosterone, progesterone, prolactin, gonadotrophins, glucose, insulin, and leptin were measured in the baseline fasting blood sample. Serum leptin concentrations were measured by enzyme linked immunosorbant assay. **Results:** Serum leptin levels in PCOS patients were significantly higher than that in the control group (P= 0.005) independently of BMI, and were significantly different between insulin resistant and non-insulin resistant obese PCOS (P= 0.044), In PCOS patients there was a positive correlation between leptin and BMI (P=0.049), and there was no correlation between leptin and other hormonal indices in PCOS patients. **Conclusions:** The study revealed that the body mass index and insulin resistance are the two main factors governing serum leptin levels.

1. Introduction

Polycystic ovary syndrome is a heterogeneous endocrine disorder that affects about one in 15 women worldwide[1]. It is the most common endocrinopathy of reproductive-aged women that manifests itself with a variety of features[2]. It is characterized by hirsutism, acne, anovulation, hyperandrogenemia, polycystic ovaries, and infertility[3]. In most cases, PCOS also involves metabolic alterations such as insulin resistance (IR), hyperinsulinemia, dyslipidemia, and obesity. Additionally, altered metabolic profiles in PCOS patients have been revealed, including the enhanced glycolysis, inhibited tricarboxylic acid cycle, and disturbed levels of amino acids[4,5]. PCOS can thus lead to an increased risk of developing type 2 diabetes mellitus and cardiovascular disease compared with the general population[6].

The impact of nutrition and energy reserves on the fertility of ruminants has been extensively described, reproduction is one of the most energy demanding endeavors of any species and, hence, tight connections between the mechanisms controlling metabolism and reproductive

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integrity have developed. Metabolic cues from peripheral tissues and environmental cues translate information reflecting fuel storage and food availability to the central regulators of reproduction, thus assuring the appropriate timing of ovulation, gestation and survival of the offspring. Sufficient energy stores are critical for the attainment of reproductive maturation and maintenance of fertility in adulthood[7]. Indeed, situations of energy depletion such as anorexia nervosa, excessive exercise, or diabetes –but also extreme energy surplus (e.g., severe obesity) – lead predominantly to delayed or absent pubertal onset in adolescents and hypogonadism in adults[8,9]. Indeed, peripheral metabolic cues and reproductive hormones may act on different targets to regulate food intake and reproduction at multiple levels[10-13].

Obesity in particular, central obesity is present in 10–65% of women with PCOS[14], although obesity is an increasingly prevalent health problem worldwide, women with PCOS have a greater risk of overweight, obesity, and central obesity[15]. and its presence worsens the associated IR and metabolic and endocrine features[16].

Obesity is defined as abnormal or excessive lipid storage. It is characterized by the increased number and volume of adipocytes. It is now recognized that the white adipose tissue is a multifunctional organ. In addition to its key role of lipid storage, it has a crucial endocrine function[17]. The effects of obesity on female reproduction have been extensively investigated. Obese women seem to have impaired reproductive potential. The adverse effects of obesity on female fertility include impaired ovulation, oocyte maturation irregular menstrual cycle, endometrial development, uterine receptivity elevated miscarriage rate, lower implantation, and lower pregnancy rates[18]. In addition, the distribution of body fat is also important as central/abdominal obesity is associated with IR and has a greater impact on fertility. The presence of obesity can also magnify IR. Obesity is thus one of the crucial parameters and an independent risk factor of PCOS, which plays an important role in the development and manifestations of the clinical, biochemical, and metabolic features of PCOS[19]. Adipose tissue has been revealed to play important roles in the regulation of many physiological processes, such as reproduction, immune response, and glucose and lipid metabolism, by secreting a variety of bioactive cytokines named adipokines that exert multiple effects at both the local and the systemic level[17].

Adipokines comprise both non adipose–specific cytokines such as retinol binding protein–4 (RBP4), lipocalin–2 (LCN2), interleukin 6 (IL6), IL1 β, and tumor necrosis factor α (TNF α), and adipose–specific cytokines or cytokines predominantly secreted by adipocytes, such as, adiponectin (APN), resistin, and leptin[20]. Leptin is a 16 kDa protein which is produced by adipocytes[21], that plays a key role in regulating energy intake and energy expenditure, including appetite and metabolism. It is one of the most important adipose derived hormones[22]. It has been associated with body mass index (BMI), insulin action, and glucose metabolism[23,24]. It is also required for male and female fertility. The deficiency of leptin or leptin receptors (LEPR) due to loss of function mutations in the corresponding genes has been linked to infertility and delayed puberty development in humans and rodents. Moreover, leptin and its receptor have been implicated in maintaining other normal female reproductive functions, including lactation, folliculogenesis, ovarian steroidogenesis, the maintenance of mammary gland morphology and function, the development of dominant follicles and oocytes, maturation endometrial development, menstrual cycle regulation [25,26] and endometrial receptivity[27-31]. Leptin signaling also contributes to the hypothalamic–pituitary–gonadal (HPG) axis. In cultured pituitary cells from female rats, leptin could induce the production and secretion of both luteinizing hormone (LH) and follicle stimulating hormone (FSH), with or without gonadotropin releasing hormone[32] . In addition, mice lacking leptin or the LEPR exhibit low LH levels and incomplete development of reproductive organs. Administration of leptin to ob/ob mice induces pubertal development and maturation of reproductive organs, increases LH secretion, and restores fertility, demonstrating the importance of leptin signaling in female reproduction [33]. Abnormal leptin levels have been reported in the peritoneal fluid in women with endometriosis[34-36]. Additionally, in women with hypothalamic amenorrhea due to energy deficiency, leptin treatment resulted in the recovery of menstruation and corrected the abnormalities in gonadal, growth hormone, and adrenal axes, further indicating the requirement of leptin in normal reproductive and neuroendocrine functions[37,38] Circulating leptin levels were increased by exogenous estradiol in women[39], and modulation of leptin activity in the hypothalamus occurs partly under the influence of estrogen. Some studies have indicated that leptin levels outside an ideal range can have a negative effect on egg quality and outcome during in vitro fertilization[40,41].

Our current understanding of the role for leptin in PCOS is far from complete. The heterogeneity of clinical manifestations of PCOS patients makes this syndrome even challenging in the field of endocrinology, metabolism, and reproduction. This study discusses the role of circulating level of leptin as a major representative of peripheral metabolic cue that may act as links between obesity and PCOS, PCOS also reciprocally influences the profile of

adipokines. Insight into the underlying mechanisms will help better understand the pathology of PCOS and identify new therapeutic target of this syndrome.

2. Materials and methods

2.1. Ethical approval

This study was approved by the ethical committee of Faculty of Medicine, Taif University and in accordance with the Declaration of Helsinki for experiments involving humans. Consent was obtained from all patients and controls.

2.2. Subjects

This study included 56 subjects who divided into two main groups; 40 women with PCOS who were admitted to the Obestatric and Gynacology Department of King Abd El-aziz Hospital in Taif-Saudi Arabia, their mean age was 34.30 ± 2.08 years, and control group which included 16 apparently healthy, obese females of comparable age and body mass index to the patients, they have regular menstrual cycles of 26-30 days, normal-sized ovary and no sign of hyperandrogenism, Both groups were exposed to detailed history with special emphasis on age and menstrual history and thorough physical examination with special stress on androgenic features, measurement of arterial blood pressure, and body mass index (BMI, kg/m^2). Body mass index (BMI) was calculated as weight (in kilograms)/ height^2 (in meters). and obese subjects was defined by BMI > 30. Patients had previously been diagnosed with PCOS in accordance with the Androgen Excess and PCOS Society criteria for the polycystic ovary syndrome which based on the presence of hyperandrogenism (clinical and/or biochemical), ovarian dysfunction (oligo-anovulation and/or polycystic ovaries), and the exclusion of related disorders[44], all of them were euthyroid and normoprolactinemic. None of them had detectable pituitary or hypothalamic dysfunction. Moreover, none had received any drugs known to interfere with hormonal concentrations for at least 3 months before the study.

2.3. Sampling

Following overnight fasting period five milliliters whole venous blood samples were withdrawn from each subject by sterile tubes on 21st day of menstrual cycle and randomely from those who have irregular cycle. After clot formation, blood samples were centrifuged for 15 minutes at 2 000×g. These samples were used for estimation of serum fasting blood glucose, insulin, FSH, LH, progesterone, prolactin, and testosterone levels. Aliquots of serum samples were stored at -80 ℃ until leptin assay.

2.4. Bio-chemical assays

Fasting blood glucose, follicle stimulating hormone (FSH), luteinizing hormone (LH), progesterone, prolactin, and testosterone levels were analyzed with Architect c16200 Integrated System (Abbott Diagnostics Europe, Wiesbaden, Germany). Serum insulin levels were determined using a two site, solid phase chemiluminescent enzyme immunometric assay (CLIA) by Siemens Immulite 1 000 immunoassay system (Siemens Healthcare Diagnostics, USA). Serum leptin concentrations were determined using a quantitative sandwich enzyme immunoassay technique (ELISA) according to the manufacturer's instructions. (Biovendor-Laboratorni Medicina a.s). The leptin assay had a lowest detection limit of 0.2 ng/mL. Intra-assay and inter-assay coefficients of variation were 5.9% and 8.6% respectively.

Insulin resistance was estimated by means of homeostasis model assessment for insulin resistance (HOMA-IR) index which is calculated by the formula: fasting insulin concentration (μIU/mL) fasting blood glucose (mmol/L)/22.5. Patients were categorized into two subgroups according to the HOMA- IR value; below and equal or above 3.

2.5. Statistical analysis[42]

All the statistical analyses were performed using IBM SPSS software package version 20.0[43]. Descriptive measures were done for each variable in every group. Data in the form of continuous variables were presented as means ± standard deviation (SD). Pearson correlation coefficient was used to determine the relationship between continuous variables. Kolmogorov-Simonov goodness of fit test was performed to evaluate normality of quantitative variable. Since, the test shows the studied variables are normally distributed, for comparing the quantitative variables in the two studied groups; independent sample *t*-test was used. *P* value less than 0.05 was considered as a statistically significant. All results are two-tailed.

3. Results

The anthropometric & laboratory data of the studied groups are summarized in (Table 1) as follows: 40 obese women with PCOS (group 1), and 16 apparently healthy obese females (group 2), the mean age of group I was 34.30 ± 2.08

Table 1

Mean age, anthropometric measurement, metabolic and hormonal data in obese PCOS women (Group 1), and obese ovulatory women (Group 2).

Variable	Group 1 (n=40)	Group 2 (n=16)	t	P value
Age (years)	34.30±2.08	28.10±4.61	2.040	0.138
BMI (kg/m^2)	34.84±4.77	33.59±1.23	2.207	0.078
Glucose (μIU/mL)	6.04±1.61	4.75±0.26	2.161	0.067
Insulin (μIU/mL)	11.02±4.79	9.30±5.71	1.018	0.343
HOMA-IR	2.96±1.43	2.12±1.44	1.671	0.139
FSH (mIU/mL)	4.80±2.58	6.80±1.73	2.320	0.049*
LH (mIU/mL)	7.71±6.91	5.14±1.65	1.177	0.027*
Testosterone (ng/mL)	0.91±0.49	0.49±0.30	2.427	0.046*
Progesterone (nmol/L)	0.90±2.01	4.30±2.10	2.161	0.045*
Prolactin (ng/mL)	15.18±10.68	13.26±1.35	0.571	0.582
Leptin (ng/mL)	23.78±5.99	16.86±0.90	3.647	0.005*

*Significant.

years, and group II was 28.10 ± 4.61 years, The mean of BMI in group I was 34.84 ± 4.77 while in group II was 33.59 ± 1.23. There was no significant difference between the mean of age and BMI between patients with PCOS and their obese controls (P= 0.138, and, P= 0.078) respectively.

There was no significant difference between the mean of serum fasting glucose levels, Insulin levels and HOMA-IR of patients with PCOS and their obese controls (P=0.067, P=0.343, P=0.139), respectivey.

Serum progesterone, and FSH were significantly lower in the study group as compared to their obese control (P=0.045, P=0.049), while LH, and testosterone levels were significantly higher than the controls (P=0.027, P=0.046). The mean leptin levels were (23.78± 5.99), and (16.86±0.90) ng/mL in the study group and their obese controls. Leptin levels were significantly higher in the study group than their obese controls (P= 0.005).

In the present study, a significant correlation was found between leptin levels and BMI (P=0.049), but no correlation was found between leptin and any of the studied parameters (table 2).

Table 2

Correlation between leptin and different parameters.

Variable	Leptin	
	r	p value
Age	0.633	0.367
BMI	0.809	0.049*
Prolactin	−0.094	0.796
Progesterone	−0.425	0.221
LH	−0.088	0.810
FSH	0.225	0.560
Testosterone	−0.119	0.780
Glucose	0.052	0.903
Insulin	0.279	0.503
HOMA	0.315	0.447

r: Pearson coefficient

The study group was further classified into two subgroups, those with insulin resistance and those without, patients with insulin resistance had significantly higher leptin levels than noninsulin resistant subgroup (P= 0.044), but no significance difference was found between the two subgroups in BMI (P=0.413) (Table 3), further more when noninsulin resistant subgroup was compared to obese control, no significant difference was found between them in leptin levels.

Table 3

BMI and serum leptin level in noninsulin resistant and insulin resistant obese women with PCOS(mean ± SD).

Parameters	Noninsulin resistant subgroup (n = 27)	Insulin resistant subgroup (n = 27)	t	P value
BMI (kg/m^2)	30.03±4.58	32.4±3.39	0.614	0.413
Leptin (ng/mL)	16.22±2.59	25.52±5.56	3.350	0.044*

4. 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. Ovary is an ever-changing tissue and dynamic multicompartmental organ, which is under the chief regulatory control of hypothalamic, pituitary principles. The hypothalamic-pituitary control over ovarian functions, however, is precisely governed by a plethora of external factors and internal peripheral principles including many of ovarian origin. Leptin has emerged as peripheral signal and a potential regulator of many reproductive functions including gametogenic and steroidogenic potential of ovary. It is considered as a possible link between nutrition

and reproduction[44].

Serum leptin was found to be keenly interrelated with estrogens, progesterone, androgens, and insulin, but their role in the regulation of circulating leptin levels is still obscure. Though leptin is widely present in reproductive tissues, its relationship to reproductive hormones is still poorly understood. Controversial results have been reported during hormone replacement therapy, oral contraceptive intake, and ovulatory disorders[45,46].

Polycystic ovarian syndrome, the common dysovulatory infertility, is characterized by chronic anovulation, hyperandrogenemia, insulin resistance, and a high incidence of obesity[47]; features which are often linked to leptin and its receptor. These facts thus make PCOS women a useful tool to assess the inter-regulatory phenomena between leptin and ovarian function.

This study aimed to investigate changes in serum leptin concentrations among obese women with PCOS and healthy obese women. This study showed no significant relationship between age and leptin concentrations. However, Zhong, *et al* [48] revealed that serum leptin concentrations varied with age. This may be explained by the fact that our study groups were at a pre-menopausal stage, whereas the study group in Zhong *et al* work consisted of both pre-and post-menopausal women, and the mean age of the study group (45.40 ± 14.80 years) was higher than that of our study groups (34.30 ± 2.08 and 28.10 ± 4.61 years).

The means of serum progesterone, and FSH were significantly lower in the study group as compared to their obese control (P=0.045, P=0.049), while LH, and testosterone levels were significantly higher than the controls (P=0.027, P=0.046), and these are considered the typical endocrine features of the PCOS.

In the present study, a significant correlation was found between leptin levels and BMI (P=0.049), and this result was in accordance with previous studies which confirm close relations between leptin and measures of fat mass and insulin sensitivity[49-53]. Leptin originates from fatty tissue; thus, an increase in body fat results in more leptin production. Therefore, our results are reasonable. Our findings of insignificant associations between leptin and androgen levels are in agreement with previous studies[52,53]. Further supporting a minor relation between testosterone and leptin, Krotkiewski *et al*[54] found no significant changes of leptin levels during anti-androgen treatment in PCOS.

In this study, the mean leptin level was 23.78 ± 5.99 ng/mL which was significantly higher in obese PCOS women than their obese control (16.86 ± 0.90 ng/mL) (P= 0.005) despite comparable BMI (P= 0.078), and this finding stimulated further search for the factors implicated in this difference. These findings were in accordance with some studies that suggested that a substantial proportion of women with PCOS have leptin levels that are higher than expected for their BMI [55], and other studies which provided evidence that circulating leptin levels are fully accounted for by the degree of adiposity and BMI compared to matching control subjects[56-58]. On the other hand, Conway and Jacobs[59], 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.

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, so 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; Poretsky and Kahin [60] 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[61], 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[62], and attenuates several insulin induced activities in hepatocytes[63]. 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 [64], furthermore, leptin treatment of these cells *in vitro*, caused significant reduction in its steroid output[65]. It is possible that leptin has a dual effect on reproduction and that the major site of action differs according to the circulating levels[66]. The positive action of leptin on hypothalamic pituitary level might be a crucial trigger 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.

Conflict of interest statement

We declare that we have no conflict of interest.

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