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# Comparison of serum irisin and leptin levels in obese and non-obese women with polycystic ovary syndrome

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**Polycystic ovary syndrome (PCOS) is a common endocrine disorder affecting reproductive-age women, often associated with obesity and metabolic dysfunction. This study investigated the relationship between two key metabolic biomarkers, irisin and leptin, in obese and non-obese women with PCOS compared to healthy controls. This prospective case-control study included 80 women, with 40 diagnosed with PCOS and 40 healthy controls. Each group was further divided into obese and non-obese subgroups, forming four groups. Serum irisin and leptin levels were measured using ELISA kits. Clinical and metabolic parameters (body mass index [BMI], insulin resistance, and fasting glucose) were analyzed, and correlations were evaluated. Leptin levels differed between groups, with lower levels observed in PCOS compared to controls. Irisin levels were significantly lower in obese subgroups, irrespective of PCOS status ($p < 0.05$). Leptin positively correlated with BMI, insulin levels, and insulin resistance, while irisin negatively correlated with BMI and insulin resistance. Obesity plays a role in modulating irisin and leptin levels in PCOS. These findings provide an integrated perspective on adipokine–myokine interactions in PCOS, rather than identifying novel biomarkers. Larger, longitudinal studies are needed to confirm these findings.**

**Keywords** Polycystic ovary syndrome, Irisin, Leptin, Obesity, Metabolic dysfunction

**Abbreviations**

| | |
|---|---|
| 17-OHPRG | 17-hydroxyprogesterone |
| ADMA | Asymmetric dimethylarginine |
| BMI | Body mass index |
| DHEA-SO4 | Dehydroepiandrosterone sulfate |
| ELISA | Enzyme-linked immunosorbent assay |
| FSH | Follicle-stimulating hormone |
| HOMA-IR | Homeostasis Model Assessment for Insulin Resistance |
| LH | Luteinizing hormone |
| PCOS | Polycystic ovary syndrome |
| RBP4 | Retinol-binding protein 4 |
| ROC | Receiver operating characteristic |
| SHBG | Sex hormone-binding globulin |
| TSH | Thyroid-stimulating hormone |

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders affecting women of reproductive age worldwide. The prevalence of PCOS varies depending on the diagnostic criteria used, ranging from 4% to 21% among all women[1,2]. The syndrome is characterized by a variety of clinical manifestations, including hyperandrogenism, oligomenorrhea or amenorrhea, infertility, and obesity[3]. Compared to the general population, individuals with PCOS are at a higher risk of developing cardiovascular disease, dyslipidemia, and abnormal glucose metabolism[4]. Studies have also shown that metabolic disorders are more frequently observed in obese women with PCOS[5].

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Irisin, a hormone first identified in 2012 in mouse skeletal muscle, plays a role in thermoregulation and has since been found to influence glucose and lipid metabolism, as well as energy homeostasis[6]. Irisin is also implicated in the pathologies of diseases closely related to obesity, such as diabetes, cardiovascular disease, and neurodegenerative disorders[6]. Through its ability to increase glucose uptake in skeletal muscle cells, irisin creates a hypoglycemic environment, making it a potential therapeutic target for patients with type 2 diabetes mellitus and insulin resistance[6]. Irisin has been increasingly investigated in metabolic disorders; however, its role in PCOS remains controversial. While some studies have reported elevated irisin levels in women with PCOS, possibly reflecting a compensatory response to insulin resistance, others have demonstrated reduced or unchanged levels, particularly when obesity is taken into account.

Leptin, a peptide hormone secreted by adipocytes, reduces appetite and increases energy expenditure via its effects on the central nervous system. Leptin also plays a significant role in the physiology of the reproductive system[7]. Numerous studies have demonstrated a strong relationship between leptin levels and obesity, and leptin has been investigated as a potential target in obesity research[7], although its precise role in reproductive and metabolic dysregulation remains incompletely understood.

Previous studies have typically examined these biomarkers in isolation or without adequate stratification for obesity, limiting the ability to disentangle their independent and combined effects. To our knowledge, studies simultaneously evaluating both irisin and leptin within a BMI-stratified PCOS and control framework are limited. This combined approach enables assessment of adipokine–myokine interactions while accounting for the confounding effect of obesity. We evaluated serum irisin and leptin levels in obese and non-obese women with PCOS and healthy controls using a BMI-stratifies design, allowing for a more integrated assessment of adipokine-myokine interactions in this population. This study aimed to compare serum irisin and leptin levels between obese and non-obese women diagnosed with PCOS who presented to the Recep Tayyip Erdoğan University Research and Training Hospital. By analyzing blood samples from these patients, we sought to clarify the relationship between obesity and these markers and explore their potential as targets for further investigation in the context of PCOS. Rather than aiming to identify novel biomarkers, this study is designed as an integrative and confirmatory analysis focusing on the interaction between adipokines and myokines within a BMI-stratified PCOS framework.

## Materials and methods

### Study design and setting

This prospective, observational, case-control study was conducted at the Recep Tayyip Erdoğan University Research and Training Hospital between December 2019 – May 2020. The aim was to compare serum irisin and leptin levels in women diagnosed with PCOS and a healthy control group, with further stratification based on obesity status.

### Study population

A total of 80 women were recruited for the study and divided equally into two main groups: 40 women diagnosed with PCOS and 40 healthy controls. A quota-based recruitment strategy was employed to achieve equal subgroup sizes ($n = 20$ per group) a priori. This stratified design was chosen to facilitate balanced comparisons between obese and non-obese subgroups within both PCOS and control populations. Consecutive eligible women presenting to the clinic were recruited until the target sample size for each subgroup was reached. Within these groups, the participants were further categorized based on their body mass index (BMI) into obese (BMI $\geq 30$ kg/m$^2$) and non-obese (BMI $< 30$ kg/m$^2$) subgroups. This stratification resulted in four distinct subgroups: obese PCOS ($n = 20$), non-obese PCOS ($n = 20$), obese control ($n = 20$), and non-obese control ($n = 20$).

The PCOS group included women aged 18–35 years with a clinical diagnosis of PCOS based on the Rotterdam criteria, which include the presence of at least two of the following: (1) oligoovulation or anovulation, (2) clinical and/or biochemical signs of hyperandrogenism (e.g., hirsutism, acne, and elevated serum testosterone levels), and (3) polycystic ovaries on ultrasonography (defined as the presence of 12 or more follicles in each ovary measuring 2–9 mm in diameter and/or increased ovarian volume $> 10$ cm$^3$). The control group was formed with women aged 18–35 years with regular menstrual cycles (21–35 days) and no clinical or biochemical signs of hyperandrogenism or polycystic ovaries on ultrasound.

The exclusion criteria were as follows: pregnancy or lactation, use of hormonal medications (including oral contraceptives) within the last six months, diagnosis of diabetes mellitus, cardiovascular diseases, thyroid dysfunction, or other systemic illnesses, presence of other endocrine disorders such as Cushing's syndrome, adrenal hyperplasia, or hyperprolactinemia, and history of smoking, alcohol, or drug abuse.

### Data collection

All participants underwent a comprehensive clinical evaluation, including the collection of demographic information such as age, ethnicity, marital status, and reproductive history. Menstrual history was documented, noting age at menarche, menstrual regularity, and cycle length. Clinical signs of hyperandrogenism were assessed, including hirsutism using the modified Ferriman-Gallwey score, with a score of $\geq 8$ indicating hirsutism. Acne and alopecia were also recorded.

Anthropometric measurements were taken, with height measured using a stadiometer and weight using a digital scale to calculate BMI (weight in kg/height in m$^2$). Waist circumference was measured at the midpoint between the lowest rib and the iliac crest, while hip circumference was measured at the widest point of the hips.

Biochemical assessments were performed after participants fasted for at least 8 h overnight. Venous blood samples were drawn between 08:00 and 10:00 a.m. to control for diurnal variations in hormone levels. Blood samples were collected between days 2 and 5 of the menstrual cycle for participants with regular cycles (control group and non-obese PCOS participants with regular cycles). For PCOS participants with oligomenorrhea or

amenorrhea, blood sampling was performed on a random day, with the absence of a dominant follicle (> 10 mm) confirmed by transvaginal ultrasound to minimize cyclical variation. After collection, blood samples were centrifuged at 3000 rpm for 10 min within 30 min of collection. Serum aliquots were stored at −80 °C until analysis. All samples underwent a single freeze-thaw cycle prior to ELISA analysis. Samples from all study groups were stored for comparable durations.

Hormonal assays included measurements of serum levels of luteinizing hormone, follicle-stimulating hormone, total and free testosterone, dehydroepiandrosterone sulfate (DHEA-SO4), 17-hydroxyprogesterone, and estradiol, using commercially available enzyme-linked immunosorbent assay (ELISA) kits (SunRed Bio, Shanghai, China). Metabolic parameters, including fasting blood glucose, insulin, and lipid profiles (total cholesterol, high-density lipoprotein, low-density lipoprotein, and triglycerides), were measured using standard automated methods (Roche Diagnostics, Mannheim, Germany). Insulin resistance was calculated using the Homeostasis Model Assessment for Insulin Resistance (HOMA-IR) index: HOMA-IR = (fasting glucose [mg/dL] × fasting insulin [μIU/mL])/405. Additionally, serum irisin and leptin levels were measured using specific ELISA kits (Irisin: SunRed Bio, Shanghai, China, catalog No: 201-12-5624; Leptin: SunRed Bio, Shanghai, China, catalog No: 201-12-4531), with all samples analyzed in duplicate and the mean values used for analysis.

## Ethical approval

This study was conducted in compliance with the principles of the Declaration of Helsinki and received approval from the Ethics Committee of Recep Tayyip Erdoğan University (approval number: 2019/188). Written informed consent was obtained from all participants before their inclusion in the study.

## Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 29.0 (IBM Corp., Armonk, NY, USA). The normality of continuous variables was assessed using the Shapiro-Wilk test. Descriptive statistics are presented as mean ± standard deviation (SD) for normally distributed variables and as median (interquartile range, IQR) for non-normally distributed variables. Categorical variables are expressed as frequencies and percentages. Comparisons between two independent groups were performed using the Independent Samples t-test for normally distributed variables and the Mann-Whitney U test for non-normally distributed variables. For comparisons across four groups stratified by PCOS status and obesity, the Kruskal-Wallis test was applied, followed by Bonferroni-corrected pairwise post-hoc comparisons to control for type I error inflation due to multiple comparisons.

Correlations between serum leptin and irisin levels and clinical, metabolic, and hormonal parameters were assessed using Spearman's rank correlation coefficient, given the non-normal distribution of the majority of variables. Correlation coefficients (r) and corresponding p values are reported.

To identify independent predictors of serum irisin and leptin levels, two complementary multiple linear regression analyses were performed using the simultaneous entry method. In the first model, serum irisin was entered as the dependent variable; in the second model, serum leptin was entered as the dependent variable. In both models, the following covariates were included: leptin or irisin (respectively), BMI, HOMA-IR, total testosterone, free testosterone, PCOS status, and age. Prior to interpretation, collinearity diagnostics were performed and variance inflation factor (VIF) values were calculated for all predictors; a VIF value exceeding 5 was considered indicative of significant multicollinearity. Model fit was assessed using the coefficient of determination ($R^2$) and adjusted $R^2$, and overall model significance was evaluated using the F-test.

To assess the risk of PCOS, univariate and multivariate binary logistic regression analyses were performed. Variables with a p value < 0.1 in univariate analysis were considered candidates for inclusion in the multivariate model. Results are expressed as odds ratios (OR) with 95% confidence intervals (CI). The discriminatory capacity of serum leptin and irisin for PCOS was evaluated using receiver operating characteristic (ROC) curve analysis. The area under the curve (AUC) with 95% CI was calculated for each biomarker. The optimal cut-off value was determined using the Youden Index (J = Sensitivity + Specificity − 1), and corresponding sensitivity and specificity values are reported.

A two-tailed p value < 0.05 was considered statistically significant for all analyses.

## Power analysis

A power analysis was conducted prior to the study to determine the sample size required to detect a significant difference in serum irisin and leptin levels between the groups, with a power of 80% and an alpha level of 0.05. Based on previous studies, an effect size of 0.64 was used to calculate the required sample size, resulting in a minimum of 40 participants main groups. The subgroup size of 20 was determined based on feasibility and the quota-based design, with the understanding that subgroup analyses would be exploratory.

# Results

Eighty women were included and stratified into four equal groups (n = 20 each): non-PCOS/non-obese, non-PCOS/obese, PCOS/non-obese, and PCOS/obese.

## Group comparisons

BMI and HOMA-IR confirmed appropriate group stratification, with significantly higher values in obese groups compared to non-obese groups (all p < 0.001), while no differences were observed within obesity-matched groups (Table 1).

LH levels were significantly higher in the PCOS non-obese group compared to non-PCOS non-obese participants (p = 0.001), whereas this difference was not observed in obese groups. Total testosterone was higher in both the PCOS non-obese and non-PCOS obese groups compared to non-PCOS non-obese participants.

| | NonPCOS/NonObese | NonPCOS/Obese | PCOS/NonObese | PCOS/Obese | p |
|---|---|---|---|---|---|
| HOMA-IR | 1.5 (0.7) | 2.75 (2.6) | 1.6 (0.8) | 3.3 (1.7) | <0.001 |
| BMI | 23.6 (4.35) | 31.8 (4.2) | 21.9 (4.4) | 33.2 (5.5) | <0.001 |
| FPG | 88.5 (6.5) | 92 (14) | 91 (9) | 96 (14) | 0.086 |
| Insulin | 7.65 (2.45) | 12.2 (9.35) | 7.5 (3.8) | 13.3 (6.7) | <0.001 |
| 17-OHPRG | 1.21 (0.5) | 1.36 (1) | 1.6 (0.7) | 1.2 (0.5) | 0.003 |
| DHEAS | 207 (96.5) | 269 (148) | 312 (189) | 169 (120) | 0.005 |
| TT | 31 (12) | 42 (15.7) | 41.4 (18) | 43 (22) | 0.012 |
| FT | 1.46 (1.8) | 1.775 (1.2) | 1.47 (1.01) | 2 (1.3) | 0.726 |
| FSH | 5 (1) | 4.6 (2) | 5 (3) | 4.6 (1.7) | 0.185 |
| LH | 3 (2.5) | 5.05 (4.45) | 8 (9) | 5.4 (4.8) | <0.001 |
| TSH | 1.675 (1.1) | 2.15 (1.3) | 1.78 (1.38) | 1.9 (1.3) | 0.268 |
| PRL | 14 (6.5) | 15.5 (10.6) | 19 (8.4) | 18 (11.8) | 0.268 |
| E2 | 31 (8.5) | 37.5 (18.5) | 36 (12) | 38 (15) | 0.074 |
| Irisin | 87.9 (274.3) | 41.4 (114.3) | 75 (110.7) | 38.1 (41.1) | 0.006 |
| Leptin | 4.6 (21.4) | 2.2 (4.5) | 4 (5.1) | 2.2 (2.5) | 0.008 |

**Table 1**. Comparison of clinical, hormonal, and biochemical parameters across four groups stratified by PCOS status and obesity (non-PCOS/non-obese, non-PCOS/obese, PCOS/non-obese, PCOS/obese). Data are expressed as Median (Interquartile Range). PCOS polycystic ovary syndrome; BMI body mass index ($kg/m^2$); HOMA-IR homeostatic model assessment of insulin resistance; FPG fasting plasma glucose (mg/dl); 17-OHPRG 17-OH progesterone (ng/ml); DHEA-S dehydroepiandrosterone sulfate (µg/dl); TT total testosterone (ng/dl); ST free testosterone (pg/ml); FSH follicle-stimulating hormone (mIU/ml); LH luteinizing hormone (mIU/ml); TSH thyroid-stimulating hormone (µIU/ml); PRL prolactin (ng/ml); E2 estradiol (pg/ml). *p value: Kruskal-Wallis test.

DHEA-SO4 and 17-OH progesterone levels were higher in the PCOS non-obese group compared to PCOS obese participants (Table 1).

Serum irisin levels differed significantly across groups ($p = 0.006$), with lower levels in the PCOS obese group compared to the PCOS non-obese and non-PCOS non-obese groups (Table 1).

Serum leptin levels also differed significantly ($p = 0.008$), with a significant difference observed only between the PCOS obese and non-PCOS non-obese groups (Table1).

## PCOS vs. control analysis

When analyzed regardless of obesity, the PCOS and control groups were comparable in age, BMI, and metabolic parameters (all $p > 0.05$). LH levels were significantly higher in the PCOS group ($p = 0.002$) (Table 2).

Serum irisin levels did not differ between groups ($p = 0.223$), while serum leptin levels were significantly lower in the PCOS group ($p = 0.037$) (Table 2).

## Logistic regression

In multivariate analysis, only oligomenorrhea (OR = 21.966, $p < 0.001$) and LH (OR = 1.236, $p = 0.030$) were independently associated with PCOS.

Neither serum irisin nor leptin was independently associated with PCOS (Table 3).

To evaluate independent predictors of serum irisin and leptin levels, two complementary multiple linear regression models were constructed.

In the first model, with irisin as the dependent variable, the model showed high explanatory power ($R^2 = 0.907$, adjusted $R^2 = 0.898$, $p < 0.001$). Serum leptin was the only significant independent predictor of irisin ($B = 12.547$, $\beta = 0.937$, $p < 0.001$). BMI had a modest but significant negative association with irisin ($p = 0.040$), while HOMA-IR, androgen levels, age, and PCOS status were not significant predictors.

In the second model, with leptin as the dependent variable, the model also showed a strong fit ($R^2 = 0.905$, adjusted $R^2 = 0.896$, $p < 0.001$). Serum irisin was the only significant independent predictor of leptin ($B = 0.071$, $\beta = 0.950$, $p < 0.001$), while BMI, HOMA-IR, androgen levels, age, and PCOS status were not independently associated.

Variance inflation factor (VIF) values were below 5 in both models, indicating no evidence of multicollinearity.

The discriminatory potential of serum irisin and leptin for PCOS diagnosis was evaluated using receiver operating characteristic (ROC) curve analysis. Serum leptin demonstrated a statistically significant, albeit modest, discriminatory ability for PCOS, with an area under the curve (AUC) of 0.638 (95% CI: 0.518–0.758, $p = 0.037$). At the optimal cut-off value of 2.6 ng/ml, leptin yielded a sensitivity of 56.3% and a specificity of 62.5% for the identification of PCOS. In contrast, serum irisin did not demonstrate significant discriminatory capacity for PCOS (AUC = 0.581, 95% CI: 0.457–0.705, $p = 0.223$) (Fig. 1).

Spearman correlation analysis was performed to evaluate the relationships between serum leptin and irisin levels and clinical, metabolic, and hormonal parameters across the entire study population (Table 4). Serum leptin demonstrated significant negative correlations with BMI and insulin. No significant correlations were

| | NonPCOS (n=40) | PCOS (n=40) | p* |
|---|---|---|---|
| Age | 24±4 | 24±4 | 0.824 |
| Age at menarche | 13±1 | 14±2 | 0.010 |
| HOMA-IR | 2.1 (1.25) | 2.2 (1.75) | 0.539 |
| BMI | 28.8±5.4 | 28.1±7 | 0.631 |
| FPG | 90 (7) | 91.5 (12) | 0.482 |
| Insulin | 9 (5.1) | 10 (7.4) | 0.429 |
| 17-OHPRG | 1.25 (0.735) | 1.385 (0.565) | 0.094 |
| DHEAS | 233 (145) | 225.5 (201) | 0.776 |
| TT | 36.65 (13.5) | 42.5 (18.5) | 0.068 |
| FT | 1.64 (1.52) | 1.615 (1.25) | 0.848 |
| FSH | 4.95 (1) | 5 (1.9) | 0.152 |
| LH | 4 (3.55) | 7 (5.15) | 0.002 |
| TSH | 1.8±0.8 | 2±0.9 | 0.384 |
| PRL | 14 (8.5) | 18.5 (10) | 0.060 |
| E2 | 32 (13) | 36.5 (13) | 0.063 |
| Irisin | 54.7 (190.1) | 54.2 (53.3) | 0.223 |
| Leptin | 3.3 (10.4) | 2.3 (3) | 0.037 |

**Table 2**. Comparison of clinical, hormonal, and metabolic parameters between women with PCOS and non-PCOS controls, irrespective of obesity status. Data are expressed as Mean ± Standard Deviation or Median (Interquartile Range) as appropriate. PCOS polycystic ovary syndrome; HOMA-IR homeostatic model assessment of insulin resistance; BMI body mass index (kg/m²); FPG fasting plasma glucose (mg/dl); 17-OHPRG 17-OH progesterone (ng/ml); DHEAS dehydroepiandrosterone sulfate (µg/dl); TT total testosterone (ng/dl); FT free testosterone (pg/ml); FSH follicle-stimulating hormone (mIU/ml); LH luteinizing hormone (mIU/ml); TSH thyroid-stimulating hormone (µIU/ml); PRL prolactin (ng/ml); E2 estradiol (pg/ml). *p value: Independent Samples t-test or Mann-Whitney U test.

| | Univariate regression | | | | Multivariate regression | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 95% CI | | | | 95% CI | |
| Variables | p | OR | Lower | Upper | p | OR | Lower | Upper |
| Oligomenorrhea (1) | <0.001 | 15.4 | 4.581 | 51.771 | <0.001 | 21.966 | 5.021 | 96.102 |
| Hyperandrogenism (1) | 0.012 | 3.358 | 1.305 | 8.641 | | | | |
| Obesity (1) | 0.855 | 0.920 | 0.376 | 2.251 | 0.157 | 0.383 | 0.102 | 1.446 |
| Age at menarch | 0.016 | 1.499 | 1.077 | 2.088 | | | | |
| Menstrual cycle interval | 0.002 | 1.050 | 1.018 | 1.082 | | | | |
| 17-OHPRG | 0.090 | 1.855 | 0.908 | 3.789 | | | | |
| TT | 0.043 | 1.035 | 1.001 | 1.071 | | | | |
| FSH | 0.065 | 1.433 | 0.978 | 2.099 | | | | |
| LH | 0.004 | 1.260 | 1.075 | 1.477 | 0.030 | 1.236 | 1.020 | 1.498 |
| İrisin | 0.147 | 0.997 | 0.994 | 1.001 | | | | |
| Leptin | 0.057 | 0.950 | 0.901 | 1.001 | | | | |

**Table 3**. Univariate and multivariate logistic regression analysis of factors associated with PCOS risk. Variables with $p < 0.1$ in univariate analysis were considered for inclusion in the multivariate model. Obesity status was forced into the multivariate model as a covariate for adjustment purposes. Results are presented as odds ratios (OR) with 95% confidence intervals (CI). PCOS polycystic ovary syndrome; OR odds ratio; CI confidence interval; TT total testosterone (ng/dl); LH luteinizing hormone (mIU/ml); FSH follicle-stimulating hormone (mIU/ml); 17-OHPRG 17-OH progesterone (ng/ml).

observed between leptin and age, menarcheal age, FPG, 17-OHPRG, DHEAS, TT, FT, FSH, LH, TSH, PRL, or E2.

Serum irisin demonstrated significant negative correlations with BMI and insulin, and a significant negative correlation with fasting plasma glucose. Additionally, irisin showed a significant positive correlation with DHEAS. No significant correlations were found between irisin and age, menarcheal age, 17-OHPRG, TT, FT, FSH, LH, TSH, PRL, or E2.

A strong positive correlation was observed between leptin and irisin ($r = 0.818$, $p < 0.001$), as shown in the scatter plot (Fig. 2).

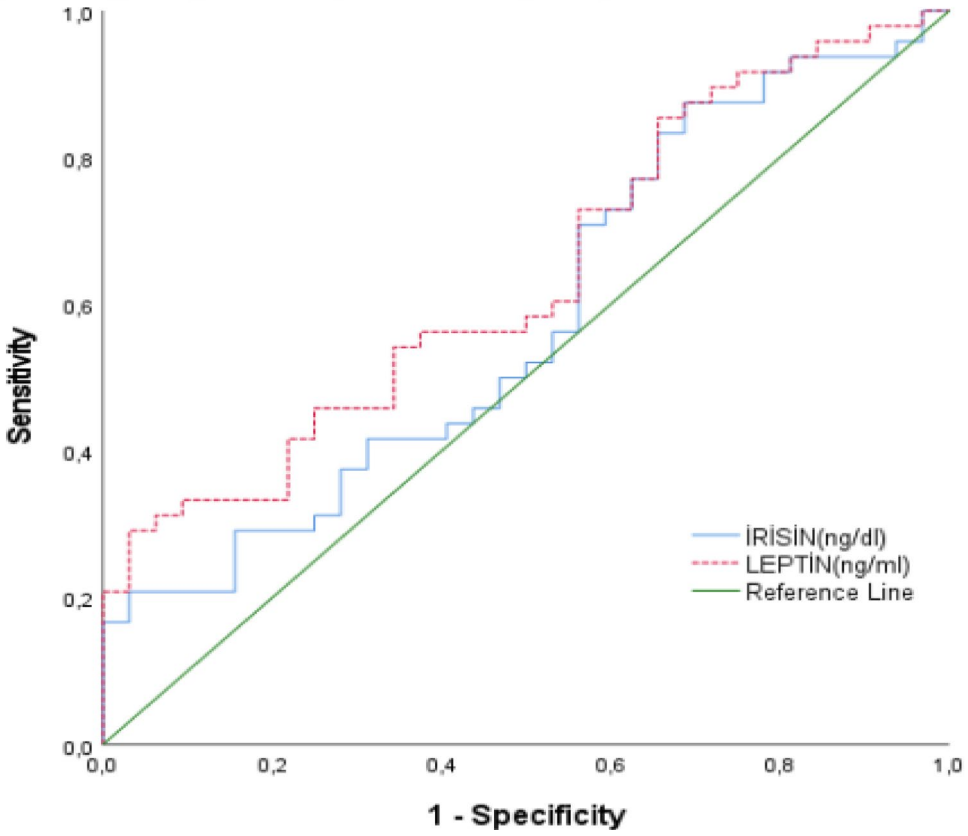



**Fig. 1**. Receiver operating characteristic curves for serum leptin and irisin as discriminators of PCOS. The diagonal reference line represents an AUC of 0.5, indicating no discriminatory ability. The optimal cut-off was determined using the Youden Index (J = Sensitivity + Specificity − 1).

## Discussion

In this study, we investigated serum levels of irisin and leptin in obese and non-obese women with PCOS and compared them to healthy controls. Importantly, evaluating adipokine–myokine interplay within a BMI-stratified design allows disentangling the relative contributions of obesity and PCOS, which have often been confounded in previous studies. Our findings provide insight into the complex interplay between these biomarkers, obesity, and PCOS. The primary novel contribution of this study lies in the simultaneous assessment of irisin and leptin in BMI-stratified PCOS and control groups. While elevated leptin and reduced irisin levels in obese women with PCOS have been reported separately, the concurrent evaluation of these two biomarkers, representing both adipose tissue and skeletal muscle endocrine functions, provides a more integrated understanding of the metabolic disturbances in PCOS. Rather than identifying entirely novel biomarkers, the present study contributes an integrative and confirmatory analysis of irisin and leptin in well-characterized, BMI-stratified PCOS and control groups.

Interestingly, in contrast to most previous studies reporting elevated leptin levels in women with PCOS, we observed lower circulating leptin concentrations in the PCOS group compared to controls in our cohort[7,8]. Importantly, this finding aligns with our correlation analyses, which demonstrated an inverse association between BMI and serum leptin levels, suggesting that the observed pattern is not incidental but reflects an internally consistent dataset. From a mechanistic perspective, several biologically plausible explanations may account for this unexpected inverse relationship. First, leptin resistance is well documented in obese women with PCOS. Chronic hyperleptinemia can lead to receptor desensitization and downregulation of leptin signaling pathways, potentially dissociating circulating leptin levels from adipose tissue mass in this population[9]. Second, androgens have been shown to suppress leptin secretion from adipose tissue. Given the hyperandrogenic environment characteristic of PCOS, the expected BMI-driven increase in leptin may be attenuated, resulting in relatively lower circulating leptin levels[10]. Finally, the relatively homogeneous nature of our study population, consisting exclusively of reproductive-age women, may have introduced range restriction, a statistical phenomenon that can attenuate or even reverse correlation coefficients. Taken together, these mechanisms provide a plausible biological and statistical framework to explain the counterintuitive inverse BMI–leptin relationship observed in our cohort. The positive correlation between leptin and HOMA-IR in our study supports the idea that leptin is associated with insulin resistance in PCOS[11,12]. Leptin levels are generally elevated in obese individuals and in women with PCOS, reflecting increased adiposity and possible leptin resistance. However, the interaction

| Parameters | Irisin | | Leptin | |
|---|---|---|---|---|
| | r | p | r | p |
| Age | -0.206 | 0.067 | -0.184 | 0.103 |
| Age at menarche | 0.020 | 0.862 | -0.099 | 0.318 |
| BMI | -0.391 | 0.000 | -0.287 | 0.010 |
| FPG | -0.249 | 0.026 | -0.214 | 0.057 |
| Insulin | -0.369 | 0.001 | -0.339 | 0.002 |
| FSH | 0.084 | 0.457 | 0.112 | 0.323 |
| LH | 0.000 | 0.998 | -0.093 | 0.414 |
| E2 | -0.115 | 0.310 | -0.157 | 0.164 |
| TSH | -0.166 | 0.141 | -0.063 | 0.577 |
| TT | 0.100 | 0.378 | -0.029 | 0.801 |
| FT | -0.076 | 0.502 | -0.040 | 0.726 |
| PRL | 0.216 | 0.265 | 0.158 | 0.163 |
| 17-OHPRG | 0.060 | 0.598 | 0.059 | 0.601 |
| DHEAS | 0.324 | 0.003 | 0.187 | 0.097 |
| FGS | 0.042 | 0.713 | -0.046 | 0.684 |
| Irisin | - | - | 0.818 | <0.001 |

**Table 4**. Spearman correlation coefficients between serum irisin and leptin levels and clinical, metabolic, and hormonal parameters in the overall study population. *BMI* body mass index (kg/m²); *FGS* Ferriman-Gallwey score; *FPG* fasting plasma glucose (mg/dl); *17-OHPRG* 17-OH progesterone (ng/ml); *DHEAS* dehydroepiandrosterone sulfate (µg/dl); *TT* total testosterone (ng/dl); *FT* free testosterone (pg/ml); *FSH* follicle-stimulating hormone (mIU/ml); *LH* luteinizing hormone (mIU/ml); *TSH* thyroid-stimulating hormone (µIU/ml); *PRL* prolactin (ng/ml); *E2* estradiol (pg/ml); *r* Spearman correlation coefficient.

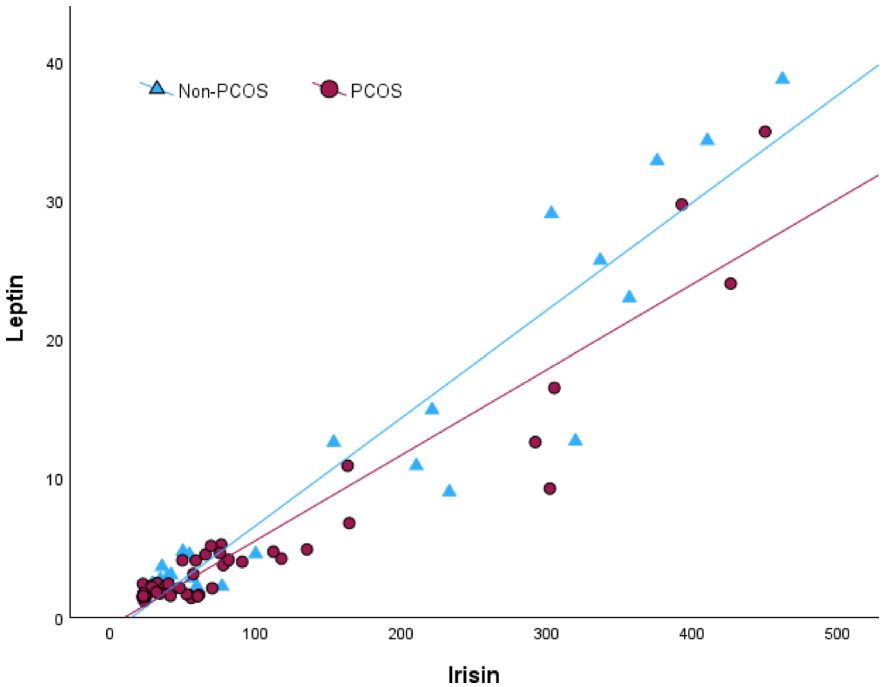



**Fig. 2**. Scatter plot depicting the correlation between serum irisin and leptin levels stratified by PCOS status. Scatter plot illustrating the relationship between serum irisin (x-axis, ng/dl) and serum leptin (y-axis, ng/ml) levels in women with PCOS (PCOS; filled circles, red regression line) and non-PCOS controls (triangles, blue regression line). Each data point represents an individual participant. Linear regression lines are fitted separately for each group. The positive association between irisin and leptin is evident in both groups; however, the regression line of the non-PCOS group demonstrates a steeper slope compared to the PCOS group, suggesting a relatively attenuated leptin response per unit increase in irisin in women with PCOS.

between leptin and reproductive function remains complex, with evidence suggesting both direct ovarian effects and indirect modulation through insulin resistance and hyperandrogenism.

Interestingly, we observed that irisin levels were lower in obese women regardless of their PCOS status, suggesting that obesity exerts a more substantial influence on irisin than PCOS itself. Irisin, a myokine produced during physical activity, has been shown to improve glucose metabolism, making it a potential therapeutic target for insulin resistance and related metabolic disorders. Our findings are consistent with those of Ali et al. and Foda et al., who also reported that irisin levels were influenced by obesity and tended to be lower in obese individuals with PCOS[13,14]. The relationship between irisin and PCOS remains controversial in the literature. While some studies have reported increased circulating irisin levels in PCOS patients, potentially reflecting a compensatory response to insulin resistance, others have demonstrated reduced or unchanged levels. These discrepancies may be explained by differences in study populations, obesity status, physical activity levels, and assay methodologies. Importantly, the strong positive association between leptin and irisin observed in unadjusted analyses remained robust after multivariable adjustment for BMI, insulin resistance, androgen levels, age, and PCOS status. This suggests that the leptin–irisin relationship is not solely driven by shared metabolic determinants such as adiposity, but may reflect a more direct or biologically interconnected adipokine–myokine axis.

The role of hyperandrogenism in PCOS cannot be overlooked. Studies have shown that elevated androgen levels are closely linked to insulin resistance and metabolic syndrome in PCOS[11]. The relationship between leptin and androgen levels in our study aligns with findings from Boyle et al. and McCartney and Marshall, who reported that hyperandrogenemia could modulate leptin sensitivity, further complicating the metabolic profile of women with PCOS[1,4]. However, in our dataset, correlations between leptin and androgen-related parameters were weak and not retained in adjusted analyses.

In this context, our findings support the notion that obesity is a dominant determinant of leptin and irisin levels, and highlight the importance of BMI stratification when interpreting these biomarkers in PCOS populations. Notably, neither leptin nor irisin emerged as independent predictors of PCOS in multivariable logistic regression analyses. While these findings provide insight into the metabolic milieu of PCOS, they should not be interpreted as evidence for therapeutic targeting. Rather, they highlight the need for further mechanistic and longitudinal studies to clarify whether these biomarkers play a causal or merely associative role.

The strengths of this study include the simultaneous investigation of two key biomarkers, irisin and leptin, in the context of PCOS and obesity, providing a comprehensive understanding of metabolic disturbances. Additionally, the comparison between obese and non-obese groups enhances the clinical relevance of the findings. The inclusion of multivariable analyses to adjust for potential confounders strengthens the validity of the observed associations.

Several limitations should be acknowledged. First, the case-control design allows inference of associations but not causality. Second, the subgroup size of 20 participants per group limits statistical power, particularly for detecting moderate or small effect sizes. Multivariable analyses with this sample size should be interpreted as exploratory. Third, menstrual cycle phase was not standardized across all participants, particularly in women with oligomenorrhea. Given that leptin levels may vary across the menstrual cycle, this may have introduced variability in leptin measurements. Fourth, although all samples were stored under identical conditions and underwent only one freeze-thaw cycle, long-term storage may affect cytokine stability, particularly for irisin. Fifth, physical activity and muscle mass, important determinants of irisin levels, were not assessed in this study. Sixth, the single-center nature of the study limits its external validity.

The findings of this study suggest several directions for future research. Larger, prospective, longitudinal cohorts with standardized protocols for sample collection and comprehensive assessment of lifestyle factors, including physical activity and diet, are needed to validate these findings. Further studies should investigate whether interventions that increase irisin levels, such as physical activity, could influence metabolic outcomes in women with PCOS. Additionally, research into leptin signaling pathways may provide insights into metabolic disturbances in PCOS.

## Conclusions

This study reinforces the complex interplay between obesity, PCOS, and metabolic biomarkers such as leptin and irisin. The elevated leptin levels and decreased irisin levels observed in obese women with PCOS underscore the importance of addressing obesity as a key factor in the metabolic phenotype of PCOS. The findings of this study should be considered exploratory and require validation in larger, prospective, longitudinal cohorts with standardized protocols for sample collection and comprehensive assessment of lifestyle factors including physical activity and diet. Future research should focus on further elucidating the mechanisms by which these biomarkers influence metabolic and reproductive health in PCOS.

## Data availability

All data supporting the results of this study are available from the corresponding author upon reasonable request.

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## Author contributions

Conceptualization, Yeliz Colakoglu and Senol Senturk; Data curation, Medeni Arpa; Formal analysis, Medeni Arpa; Funding acquisition, Senol Senturk; Investigation, Yeliz Colakoglu and Senol Senturk; Project administration, Senol Senturk; Resources, Senol Senturk; Supervision, Senol Senturk; Validation, Medeni Arpa; Visualization, Yeliz Colakoglu and Senol Senturk; Writing—original draft, Yeliz Colakoglu, Senol Senturk and Alperen Aksan; Writing—review & editing, Yeliz Colakoglu, Senol Senturk and Alperen Aksan. All authors have read and agreed to the published version of the manuscript.

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Statement:

Informed Consent Statement:

## Declarations

## Competing interests

The authors declare no competing interests.

## Informed consent

Written informed consent was obtained from all participants before their inclusion in the study.

## Institutional review board

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Recep Tayyip Erdoğan University (protocol code: 2019/188).

## Additional information

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