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Original Article

The Comparison of Irisin, Subfatin, and Adropin in Normal-Weight and Obese Polycystic Ovary Syndrome Patients

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Abstract

Background: A combination of genetic and environmental factors contribute to the highly common, complex, and varied endocrine condition known as polycystic ovary syndrome (PCOS) in women. PCOS primarily affects women between the ages of 15 and 35 who are in the early to late stages of pregnancy. Thus, this study aimed to evaluate the serum levels of irisin, subfatin, and adropin in PCOS with and without obesity compared to the control group.

Methods: The present cross-sectional study was conducted in 2022 at Al-Nahrain University/Department of Chemistry (Baghdad, Iraq). The serum levels of irisin, subfatin, and adropin were measured with the enzyme-linked immunosorbent assay (ELISA) method. Body mass index, lipid profile, insulin, fasting glucose, follicle-stimulating hormone, and luteinizing hormone levels were also evaluated. The data were analyzed using one-way analysis of variance (ANOVA) by GraphPad Prism software version 8.0.2. A $P<0.05$ was considered statistically significant.

Results: The study population comprised PCOS patients (n=90, divided into 45 obese and 45 normal weight) and healthy women (n=30). According to the results, the serum levels of irisin were significantly higher ($P<0.001$) in obese and normal-weight PCOS patients than controls. While adropin and subfatin were significantly lower in PCOS than controls ($P<0.001$). Moreover, there are higher levels of serum insulin, fasting glucose, and luteinizing hormone in PCOS women than in healthy women.

Conclusion: According to the findings, PCOS patients had a higher level of irisin than the controls. In addition, decreased subfatin and adropin levels were observed in PCOS patients compared with healthy women. Further research is required to confirm these results in the future.

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Keywords ● Polycystic ovary syndrome ● Hyperandrogenism ● Infertility ● Menstruation disturbances ● Obesity

What's Known

- Obesity is associated with polycystic ovary syndrome (PCOS) development and contributes to metabolic abnormalities in women with PCOS.

What's New

- In PCOS, serum irisin levels were significantly increased. In addition, PCOS was associated with lower levels of subfatin and adropin than controls.
- Changes in the serum concentrations of subfatin, irisin, and adropin may serve as PCOS biomarkers.

Introduction

Polycystic ovary syndrome (PCOS) is the most prevalent cause of infertility, affecting approximately 9–18% of women of reproductive age.[1] Significant metabolic and reproductive disturbances, including oligo/anovulatory cycles, infertility, insulin resistance, dyslipidemia, obesity, and type 2 diabetes (T2DM), are associated with PCOS.[2] Women with a history of weight

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gain frequently experience oligomenorrhea and hyperandrogenism, indicating that obesity plays a pathogenetic role in the development of PCOS. Multiple secretory peptides and proteins (adipokines) derived from adipose tissue, including irisin, chemerin, and leptin, have been identified, and dysregulated production and function of these adipokines are associated with metabolic diseases.[3] White adipose tissue (WAT) and brown adipose tissue (BAT) are two types of adipose tissue that secrete distinct groups of adipokines. Insulin resistance (IR) and altered adipocytokine activities released by adipose tissue have been recently investigated as potential contributors to the underlying pathophysiology of PCOS.[4] Subfatin, also known as Metrnl (Meteorin-like), is a newly discovered immunoregulatory adipokine that is extensively secreted by adipose tissue and skeletal muscles.[5] Acute exposure to exercise and cold can cause white adipose tissue to express subfatin. In addition, elevated subfatin levels promote energy expenditure and enhance glucose tolerance.[6] There have only been a few research that has looked into subfatin and PCOS. Irisin is a recently discovered muscle-derived myokine composed of 112 amino acids, which serves as a messenger from skeletal muscle to other parts of the body.[7] Fibronectin type III domain-containing protein 5 (FNDC5) was determined to be the precursor of irisin after being cleaved. A prohormone, encoded by the *FNDC5* gene, is converted into irisin by post-translational modification.[8] Several tissues, including adipose tissue, the liver, the cardiovascular system, the pancreas, the kidney, the brain, and the bone, produce irisin.[9] Therefore, the function of irisin in the development of PCOS remains unknown. Adropin is a peptide composed of 76 amino acids, which is primarily produced in the liver and brain. Adropin is a secretory protein, which is encoded by the energy homeostasis-associated (*Enho*) gene and plays important functions in insulin sensitivity and energy regulation.[10] Moreover, alterations in the expression and levels of adropin have been linked to PCOS, with decreased adropin levels resulting in impaired dyslipidemia, fasting glucose, and insulin resistance.[11] To the best of our current understanding, there have been limited studies investigating the association between subfatin, irisin, and adropin levels in individuals with PCOS. Therefore, the purpose of the present study was to investigate the levels of subfatin, irisin, and adropin in obese and normal-weight PCOS, and to evaluate the correlations between these serum levels with other parameters in PCOS.

Patients and Methods

This study was approved by the Ethics Committee of Al-Nahrain University, Baghdad, Iraq (code: 2013.2.).

This is a cross-sectional study that was conducted between 1 August 2022 and 1 December 2022 at a private clinic and Al-Yarmouk Teaching Hospital in Baghdad. The study population consisted of 90 PCOS patients divided into two groups (obese and normal weight) and 30 control cases. PCOS was diagnosed according to the revised Rotterdam criteria by the presence of at least two of three symptoms (oligomenorrhea or amenorrhea, clinical and/or biochemical hyperandrogenism, and polycystic ovary morphology on ultrasonography).[12] Patients suffering from cardiovascular diseases, diabetes, hyperprolactinemia, congenital adrenal hyperplasia, androgen-secreting tumors, chronic smoking, renal impairment, thyroid disorders, hypercholesterolemia, and hypertension, and those taking drugs such as metformin, gonadorelin, dopamine, and contraceptive pills were excluded from the study. Body mass index (BMI) (Kg/m^2) was calculated by the standard equation (BMI=weight in Kg/height in m^2). The sample size calculation was done by using a *t* test in the G* Power program (Germany 2019). The minimum sample size was found to be 24 in each group when using 80% statistical power, d=80%, and 5% type I error (α) in Iraqi women with PCOS.[13]

Sample Collection Procedures

All samples were collected during the early follicular phase (days 2-4 of the spontaneous cycle) after an overnight fasting (10-12 hours). After centrifugation to acquire serum, all serum samples were stored at -20°C until analysis. All samples were collected in a vacutainer tube containing gel. The ethical protocols for the study were supervised by the Department of Chemistry, College of Science, Al-Nahrain University in Baghdad, and the patient's verbal consent was written and documented in the questionnaire.

Serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and insulin were measured using the electrochemiluminescence immunoassay "ECLIA" technique and were operated on cobas E411 immunoassay analyzers (Roche and Hitachi Companies, Germany). Fasting serum glucose and lipid profile were measured by the spectrophotometer technique using a kit provided by Biosystem Company (Spain). The concentrations of serum irisin, subfatin, and adropin were measured using an enzyme-linked immunosorbent assay (ELISA) (MyBioSource company, United States). The Homeostasis Model

of Assessment-Insulin Resistance (HOMA-IR) index for the assessment of insulin resistance was calculated according to the following formula:
HOMA-IR=glucose (mg/dL)_insulin (IU/mL)/405

HOMA-IR≥2.5 is considered as insulin resistant.[14]

Statistical Analysis

Data were analyzed using GraphPad Prism Software, version 8.0.2 (San Diego, California, USA). The mean values from three or more groups were compared using a one-way analysis of variance (ANOVA). A *post hoc* test (Tukey's test; performed after ANOVA) was used to determine the statistical significance of the difference between the two groups. Results were presented as mean±SD. A P<0.05 was considered statistically significant. The Pearson correlation coefficient was used for testing the relationship between subfatin, irisin, and adropin with all parameters.

Results

The descriptive data for the PCOS and the control groups are summarized in table 1. Obese PCOS patients had a higher BMI than PCOS patients of normal-weight and control subjects. Furthermore,

Table 1: Descriptive data and biochemical analysis of women with polycystic ovary syndrome (PCOS) and healthy individuals

Variables	Control group (n=30) (mean±SD)	Normal-weight PCOS group (n=45) (mean±SD)	Obese PCOS group (n=45) (mean±SD)	P value (*post hoc* test)	P value (ANOVA)
Age (year)	26.20±5.786	25.53±5.57	23.85±6.682	0.9032[a] 0.4841[b] 0.2964[c]	0.24
BMI (Kg/m^2)	23.34±2.038	22.30±2.032	30.43±1.548**[b,**c]	0.079[a] <0.001[b] <0.001[c]	<0.001
TC (mg/dL)	179..6±14.98	181.1±18.3	227.3±22.37**[b,c]	0.9448[a] 0.001[b] <0.001[c]	<0.001
TG (mg/dL)	97.57±10.87	101.4±13.52	151.3±17.78**[b,**c]	0.535[a] <0.001[b] <0.001[c]	<0.001
HDL (mg/dL)	47.86±5.57	45.82±5.529	38.74±4.446**[b,**c]	0.2599[a] <0.001[b] <0.001[c]	<0.001
LH (mIU/mL)	4.668±0.9571	9.883±2.628**[a]	8.448±2.21**[c]	<0.001[a] 0.141[b] <0.001[c]	<0.001
FSH (mIU/mL)	7.624±0.9717	5.224±0.8459**[a]	5.165±0.9166**[c]	<0.001[a] 0.9538[b] <0.001[c]	<0.001
LH/FSH	0.6223±0.156	1.924±0.496**[a]	1.69±0.5597**[c]	0.001[c] 0.0935[b] 0.001[c]	<0.001
FBG (mg/dL)	83.06±4.187	87.42±6.397*[a]	93.65±8.274**[b,**c]	0.0283a <0.001[b] <0.001[c]	<0.001
Insulin (µIU/mL)	8.359±0.8538	18.61±4.131**[a]	29.38±7.721**[b,**c]	0.001[a] <0.001[b] <0.001[c]	<0.001
IR-HOMA	1.742±0.2532	4.039±1.094*[a]	6.983±2.416**[b,**c]	<0.001[a] <0.001[b] <0.001[c]	<0.001
Adropin (pg/mL)	422±54.87	126.8±22.76**[a]	100.8±10.21**[b,**c]	<0.001[a] 0.0.05[b] <0.001[c]	<0.001
Subfatin (ng/mL)	82.76±5.177	74.11±3.529**[a]	66.3±5.541**[b,**c]	<0.001[a] <0.01[b] <0.001[c]	<0.001
Irisin (ng/mL)	11.8±2.129	26.61±4.147**[a]	35.11±7.972**[b,**c]	<0.001[a] <0.001[b] <0.001[c]	<0.001

Significant *P<0.05; highly significant **P<0.001; no significant P>0.05; [a]Difference between PCOS normal weight and Controls; [b]Difference between Obese PCOS and normal-weight PCOS; [c]Difference between Obese PCOS and controls; BMI: Body mass index; TC: Total cholesterol; TG: Triglyceride; HDL: High-density lipoprotein; LH: Luteinizing hormone; FSH: Follicle stimulating hormone; IR-HOMA: Homeostatic model assessment for insulin resistance

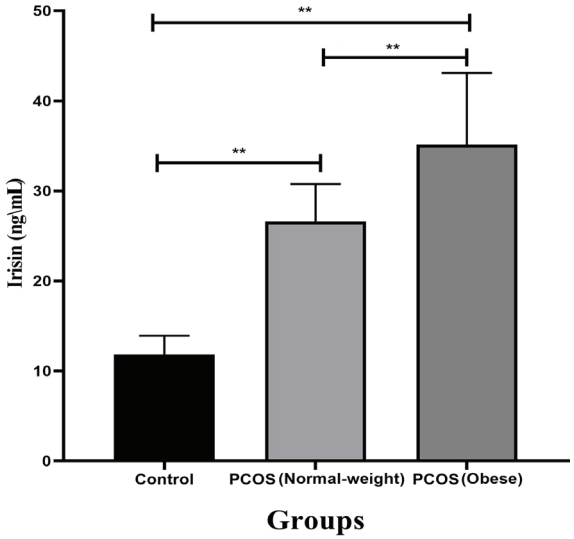

Figure 1: The Irisin level in PCOS patients and control groups. Data were analyzed using a one-way ANOVA test and represented as mean±SD. P≤0.05 is considered significant. **P<0.001; The serum level of irisin in PCOS (obese) is significantly higher than the control and PCOS (normal-weight) groups.

as shown in figure 1, the serum level of irisin increased significantly (P=0.001) in the obese PCOS group (35.11±7.972 ng/mL) compared with the normal-weight PCOS and controls (26.61±4.147 and 11.8±2.129 ng/mL, respectively). The serum levels of adropin and subfatin were significantly lower (P=0.001) in PCOS patients than in the control groups, as shown in figures 2 and 3, respectively. Moreover, normal-weight PCOS patients had significantly higher levels of insulin (18.61±4.131 µIU/mL, P=0.001) and HOMA-IR (4.039±1.094, P=0.0001) than the control groups (8.359±0.8538 µIU/mL and 1.742±0.2532 µIU/mL, respectively). Table 1 displays the lipid profile levels of the studied groups.

The levels of cholesterol and triglycerides (TG) were significantly higher in obese PCOS group (227.3±22.37 and 151.3±17.78 mg/dL, respectively) than normal-weight PCOS patients (181.1±18.3, P=0.001 and 101.4±13.52 mg/dL, P=0.0001, respectively) and controls (179.6±14.98, P=0.0001 and 97.57±10.87, P=0.0001 mg/dL respectively). Whereas high-density cholesterol (HDL) levels were lower in obese PCOS patients (38.74±4.446 mg/dL) than in control subjects (47.86±5.57 mg/dL, P=0.0001) and PCOS patients of normal weight (45.82±5.529 mg/dL, P=0.001). On the other hand, the levels of glucose, FSH, LH, and LH/FSH were significantly different in the group of obese PCOS patients when compared with the group of normal-weight PCOS patients.

The correlation between irisin, subfatin, and adropin levels and other PCOS parameters is shown in table 2. In normal-weight groups, serum levels of irisin had a significant positive

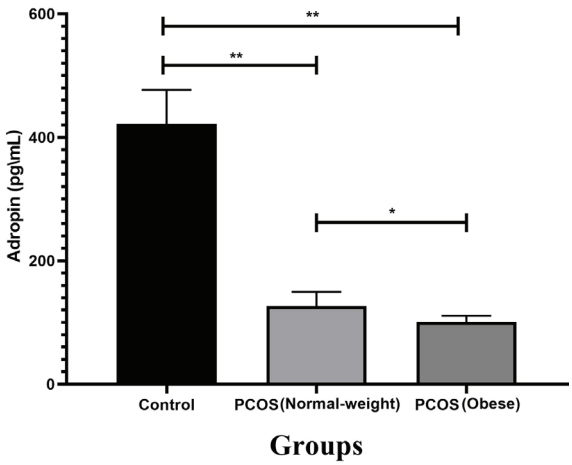

Figure 2: The adropin level in PCOS patients and control groups. Data were analyzed using a one-way ANOVA test and represented as mean±SD. *P≤0.05 is considered significant. **P<0.001; The serum level of adropin in the control group is significantly higher than in the obese and normal-weight PCOS patients.

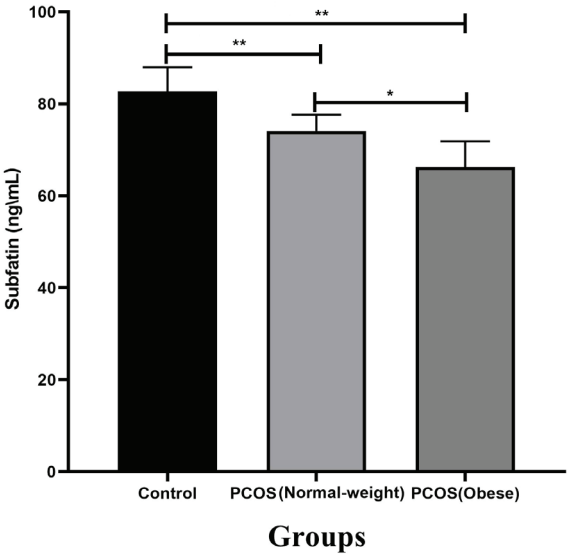

Figure 3: The Subfatin level in PCOS patients and control groups. Data were analyzed using a one-way ANOVA test and represented as mean±SD. P≤0.05 is considered significant. *P<0.01; **P<0.001; The serum level of subfatin in the control group is significantly higher than in obese and normal-weight PCOS groups.

correlation with TG, glucose, IR-HOMA, and BMI, while there was a significant negative correlation with adropin. On the other hand, subfatin levels had a negative correlation with TG. Moreover, adropin levels had a negative correlation with glucose. In the obese group, serum levels of irisin showed a significant positive correlation with TG, BMI, glucose, and IR-HOMA, while there was a significant negative correlation with adropin and subfatin. However, there was a significant negative correlation between serum subfatin level and TG, BMI, insulin, IR-HOMA, and glucose. Moreover, a significant negative correlation was observed between adropin and TG.

Table 2: Pearson correlation analysis of irisin, subfatin, and adropin with other parameters in normal-weight and obese polycystic ovary syndrome patients

Variables	Subfatin (ng/mL)		Irisin (ng/mL)		Adropin (pg/mL)	
	r	P value	r	P value	r	P value
Normal-weight PCOS						
Age (year)	0.194	0.272	-0.217	0.21	0.174	0.317
BMI (Kg/m^2)	-0.09	0.613	0.441	0.008**	0.022	0.898
TC (mg/dL)	-0.011	0.956	0.315	0.096	-0.322	0.08
TG (mg/dL)	-0.638	<0.001**	0.485	0.007**	-0.292	0.124
HDL (mg/dL)	-0.039	0.834	-0.278	0.13	0.286	0.119
LH (mIU/mL)	-0.166	0.371	0.197	0.288	-0.146	0.434
FSH (mIU/mL)	-0.261	0.156	0.155	0.404	-0.246	0.182
LH/FSH	0.09	0.631	0.105	0.574	-0.034	0.856
FBG (mg/dL)	-0.033	0.852	0.74	<0.001**	-0.443	0.009**
Insulin (µIU/mL)	-0.154	0.384	0.213	0.226	-0.218	0.215
IR-HOMA	-0.132	0.456	0.396	0.02*	-0.302	0.083
Subfatin (ng/mL)	1	---	-0.25	0.155	0.158	0.373
Irisin (ng/mL)	-0.25	0.155	1	---	-0.483	0.003
Adropin (pg/mL)	0.158	0.373	-0.483	0.003**	1	<0.0001
Obese PCOS						
Age (year)	0.033	0.846	-0.164	0.331	-0.108	0.523
BMI (Kg/m^2)	-0.458	0.004**	0.497	0.002**	-0.51	0.763
TC (mg/dL)	0.059	0.75	0.092	0.618	-0.071	0.699
TG (mg/dL)	-0.408	0.02*	0.549	0.001**	-0.439	0.012*
HDL (mg/dlL)	0.251	0.166	0.078	0.673	0.09	0.629
LH (mIU/mL)	-0.11	0.55	0.312	0.083	-0.149	0.416
FSH (mIU/mL)	-0.152	0.407	0.014	0.938	0.089	0.627
LH/FSH	0.031	0.866	0.267	0.139	-0.141	0.44
FBG (mg/dL)	-0.948	<0.001**	0.395	0.036*	-0.1	0.558
Insulin (µIU/mL)	-0.869	<0.001**	0.313	0.059	-0.068	0.69
IR-HOMA	-0.895	<0.001**	0.364	0.027*	-0.099	0.561
Subfatin (ng/mL)	1	---	-0.418	0.01**	0.064	0.705
Irisin (ng/mL)	-0.418	0.01**	1	---	-0.341	0.039*
Adropin (pg/mL)	0.064	0.705	-0.341	0.039*	1	<0.0001

Significant: *P<0.05; High Significant: **P<0.001; No significant: P>0.05; BMI: Body mass index; TC: Total cholesterol; TG: Triglyceride; HDL: High-density lipoprotein; LH: Luteinizing hormone; FSH: Follicle stimulating hormone; IR-HOMA: Homeostatic model assessment for insulin resistance

Discussion

The findings of the present study indicated that PCOS patients had a higher level of irisin than healthy women. In addition, the adropin and subfatin levels in PCOS patients were significantly lower than those in the control group. These results are in agreement with some of the previous studies,[15, 16] which will be discussed later. Irisin is a recently identified myokine involved in the brown-fat-like development from white fat, energy expenditure, weight loss, and enhanced glucose tolerance in humans.[17] Irisin and insulin resistance are still not completely understood, and it is yet unknown how this myokine is regulated. Li and colleagues demonstrated that irisin levels were significantly higher in PCOS patients than in the healthy group, as well as in overweight and obese patients compared to lean women in both groups.[18] Serum irisin was found to be positively correlated with both insulin resistance and cholesterol. Moreover, Li and colleagues showed that in overweight and obese PCOS women with a high free androgen index (FAI), higher irisin levels predict insulin resistance, metabolic syndrome, and hyperandrogenemia, further supporting the suggestion that irisin plays a prognostic role in these conditions.[19] In the present study, serum irisin levels were compared in PCOS patients and healthy individuals. Serum irisin levels were significantly different between PCOS patients and controls. Chang and colleagues were the first to report on the circulating level of irisin in PCOS, concluding that irisin may contribute to the development of PCOS and may represent a novel PCOS biomarker.[20] Higher levels of circulating irisin in patients with PCOS were also observed in several following studies.[20, 21] Another study, however, found no difference in irisin levels between PCOS and healthy women,[22] whereas in another study, patients with PCOS had significantly lower mean circulating irisin levels than controls.[23] Therefore, it was necessary to compare the levels of

circulating irisin in PCOS and healthy women. It was observed that patients with PCOS had higher irisin levels than healthy controls. Importantly, obese PCOS patients had significantly higher levels of circulating irisin than normal-weight PCOS patients and controls.[23] Numerous studies have shown a correlation between circulating irisin levels and BMI in both healthy individuals and PCOS patients.[24] In individuals with PCOS, irisin concentrations were shown to be significantly higher in obese women than in women of normal weight. Weight loss led to a significant decrease in serum irisin, while regaining lost weight restored irisin levels to their initial levels. Consequently, the current controversial results of irisin level in PCOS may be associated with the various BMI levels at baseline. In PCOS patients, the elevated irisin level may serve as a feedback mechanism to maintain metabolic balance. Additionally, irisin resistance may exist in PCOS, similar to insulin and leptin resistance observed in obesity and T2DM.[25] In most studies, women with PCOS had a higher BMI than controls. However, it is unclear whether or not the increased fat mass seen by these individuals really causes a corresponding rise in irisin production. In the present study, irisin levels were positively correlated ($P \leq 0.05$) with TG, FBG, IR-HOMA, and BMI in both obese and normal-weight PCOS. Some investigations found a correlation between irisin levels and BMI. Moreover, irisin has a positive correlation with insulin resistance in PCOS due to decreased sensitivity to irisin resistance, which results in increased irisin levels as a compensatory mechanism.[26] Adropin is encoded by the *Enho* gene and was first isolated from liver and brain tissues. Its production is associated with insulin resistance, energy homeostasis, and lipid metabolism and is hypothesized to be influenced by macronutrient consumption.[27] In our study, it was shown that adropin levels were significantly lower in women with PCOS than in healthy women. In addition, in obese patients with PCOS, the prevalence was significantly lower than in patients without PCOS. In addition, previous investigations reported that women with PCOS had reduced serum adropin levels.[28, 29] Varikasuvu and colleagues have also summarized the association between adropin levels and PCOS. They noted that PCOS patients had significantly lower adropin levels than non-PCOS controls.[30] While Kuliczkowska and colleagues found that PCOS patients had decreased serum adropin levels, there was no statistically significant difference between the healthy and patient groups.[31] In women with PCOS, obesity is more prevalent, leading to impaired glucose tolerance and insulin resistance. PCOS itself is a complex entity, and numerous metabolic factors have been associated with PCOS, so the mechanisms governing adropin production in PCOS women are not completely understood at present. Globally, the proportion of PCOS-afflicted women who are overweight varies significantly.[32] On the other hand, increasing visceral or abdominal adiposity has been associated with higher insulin resistance, which may have an effect on metabolic abnormalities in women with PCOS.[33] In addition, in a mouse model of diet-induced obesity, skeletal muscle (DIO), exogenous adropin activated insulin signaling via increasing Akt phosphorylation and glucose transporter 4 (GLUT4) translocations.[34] Subfatin is a newly discovered adipokine that shows high levels of expression in the subcutaneous fat of both rodents and humans. In addition to being found in the blood, it is also found in skeletal muscles after exercise and in adipose tissues after cold exposure. In obese/diabetic mice, increasing circulating levels of subfatin stimulated energy expenditure and gene expression associated with anti-inflammatory cytokines, as well as improved glucose tolerance.[35] Additionally, the results of this study revealed a significant decrease in the mean value of serum subfatin levels in women with PCOS than controls. This finding is in agreement with the result previously reported by Fouani and colleagues who found serum subfatin was lower in PCOS individuals than in controls.[36] Through the peroxisome proliferator-activated receptor gamma (PPAR) pathway, subfatin increases insulin sensitivity. However, the results of T2DM and obesity studies involving insulin resistance such as PCOS and subfatin were inconsistent.[37] Additionally, another study found that subfatin decreased significantly in PCOS patients and correlated negatively with the insulin resistance marker.[38] Few studies indicated the variation in subfatin levels among PCOS patients. However, when PCOS and TD2M are recognized as a group of disorders in which insulin resistance might arise, we would like to compare our results for subfatin levels to those described in TD2M. Serum subfatin levels decreased and illustrated an adverse correlation with glucose and insulin resistance in a study of newly diagnosed TD2M.[39] Consistent with previous research, this study found significantly lower levels of serum subfatin in PCOS (obese) individuals than other groups.[36] Lower circulating subfatin concentrations have been linked to obesity in previous research.[40] However, other studies have produced contradictory results revealing no correlation between serum subfatin and BMI.[41, 42] On the other hand, the relationship

between serum subfatin and obesity biomarkers remains paradoxical. Additionally, some biochemical characteristics were found to correlate with subfatin. Fasting glucose, insulin, HOMA-IR, TG, BMI, and irisin levels were all negatively correlated with subfatin concentration in obese PCOS. The results of this research are the first to suggest that PCOS patients had lower serum levels of subfatin in Iraq. This study also had several limitations such as a relatively small sample size and including only samples of Iraqi women. The percentage of body fat and skeletal muscle mass should also be assessed in future studies. Moreover, no studies were found about the relation of subfatin with PCOS to understand why subfatin decreased in PCOS.

Conclusion

The findings revealed that serum levels of irisin were significantly increased in patients with PCOS. Moreover, decreased subfatin and adropin levels were observed in PCOS compared with controls. Obesity is connected with a lower adropin and subfatin level, according to the current study. In addition, increased insulin levels may contribute to the development of insulin resistance in PCOS patients. Finally, more research is required to investigate the role of irisin, adropin, and subfatin in PCOS-related disorders associated with insulin resistance.

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Authors' Contribution

AA.M, A.H.A, and W.A: Study design; data acquisition; data analysis and interpretation; drafting and critical reviewing of the manuscript. All authors read and approved the final manuscript version and agree with all parts of the work in ensuring that any queries about the accuracy or integrity of any component of the work are appropriately investigated and handled.

Conflict of Interest: None declared.

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