

Investigating the relationship of serum levels of afamin and interleukin-10 with insulin resistance in infertile women with polycystic ovary syndrome

Tuğba Gürbüz¹, Oya Gökmen¹, Ergül Demircivi Bor², Aygül Uluocak³

¹ Department of Gynecology & Obstetrics, Medistate Kavacak Hospital, Istanbul, Turkey

² Department of Gynecology & Obstetrics, Istanbul Medeniyet University Goztepe Training and Research Hospital, Istanbul, Turkey

³ Department of Biochemistry Clinic, Medistate Kavacak Hospital, Istanbul, Turkey

ORCID ID of the author(s)

TG: 0000-0003-3555-3767
OG: 0000-0003-0020-4233
EDB: 0000-0002-4448-3863
AU: 0000-0002-0417-2358

Corresponding Author

Tuğba Gürbüz

Ruzgarlıbahçe neighbourhood Cumhuriyet street No:24, Department of Gynecology & Obstetrics, Medistate Kavacak Hospital, Istanbul, Turkey
E-mail: drtgurbuz@hotmail.com

Ethics Committee Approval

The study was conducted with the permission of the Research Ethics Committee Istanbul Medeniyet University Goztepe Training and Research Hospital (Permission granted/CAAE number: 02.12.2020, Decision no: 2020/0703). All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

Conflict of Interest

No conflict of interest was declared by the authors.

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Abstract

Background/Aim: The prevalence of Polycystic Ovary Syndrome (PCOS) is between 6-10% globally, and more than 50% of patients with PCOS are obese. The development of insulin resistance (IR) and hyperinsulinemia due to overweight can contribute to this syndrome's clinical complications. This study aimed to investigate the relationship between serum levels of afamin and Interleukin-10 (IL-10) with IR in infertile women with PCOS.

Methods: Eighty-eight participants between the ages of 18 and 36 years, with at least one year of unsuccessful attempts to have children were included in this prospective case-control study. The PCOS and healthy controls were divided into two groups based on IR. Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), Estradiol (E2), Prolactin, Thyroid Stimulating Hormone (TSH), Free T4, Anti-Mullerian Hormone (AMH), Hemogram, total cholesterol, LDL, HDL, Triglyceride, fasting blood glucose, fasting insulin, Homeostatic Model Assessment for Insulin Resistance (HOMA-IR), afamin, IL-10 values were measured.

Results: The body mass indexes (BMIs) of the groups were significantly different ($P<0.001$); and the highest BMI was observed in the PCOS group with IR ($\text{HOMA-IR} \geq 2.5$). Fasting insulin was significantly higher in the two groups with IR: 15.3 (6.09) in PCOS patients and 12.7 (2.8) in patients without PCOS ($P=0.02$). There was a weak positive correlation between BMI and Afamin ($P=0.005$), a strong negative correlation between BMI and IL-10 ($P<0.001$), a moderate negative correlation between Waist Hip Ratio (WHR) and IL-10 ($P<0.001$), a weak negative correlation between fasting insulin and IL-10 ($P=0.001$), and a weak positive correlation between monocyte and IL-10 ($P=0.03$).

Conclusion: Our results showed an increase in Afamin and a decrease of interleukin-10 in infertile women with polycystic ovary syndrome and insulin resistance.

Keywords: Polycystic ovary syndrome, Insulin resistance, Afamin, IL-10

Introduction

Polycystic ovary syndrome (PCOS) manifests in women of reproductive age and is the most important cause of infertility due to lack of ovulation [1]. It begins after puberty, and the maximum age of onset is 20 to 40 years [2]. The prevalence of this syndrome is about 6-10% [4]. More than 50% of patients with PCOS are obese [3, 4]. Body fat usually accumulates centrally and an increase in the waist-to-hip circumference ratio (WHR) increases the risk of cardiovascular diseases and diabetes mellitus (DM) [1-3].

Although obesity is not an essential feature or an inherent defect of PCOS [5], the development of hyperinsulinemia and Insulin Resistance (IR) due to overweight can contribute to the clinical complications of this syndrome [5-7]. About 50-70% of women with PCOS have IR to some degree [7, 8], which may result from obesity or associated with hyperandrogenism [9]. It has been suggested that IR can also play a significant role in the pathogenesis of PCOS [7-9].

Studies show that Afamin increase is correlated with metabolic syndrome in PCOS patients [10-12]. Afamin was first identified in 1994 as the fourth member of the human albumin gene family, including vitamin D binding protein, alpha-fetoprotein, and human serum albumin [11]. As an 87 kDa human plasma glycoprotein, afamin has 55% amino acid sequence similarity to albumin and includes 15% carbohydrate [12]. Studies have shown that elevated levels of the human afamin gene increase the body weight, glucose, and fat concentration in the bloodstream [13-15]. Some other studies have also shown an association between afamin, IR and metabolic syndrome [16-18].

Chronic inflammation in the body can be considered a stimulus for IR and type 2 DM [19]. Studies have shown that various adipose tissue hormones, such as interleukin-10 (IL-10), can significantly inhibit the expression and synthesis of proinflammatory cytokines [19-21]. IL-10 is an essential anti-inflammatory cytokine that can limit the inflammatory response caused by tissue damage [20]. IL-10 also prevents the exacerbation of inflammation by reducing inflammatory responses and suppresses the production of cytokines such as interleukin-6 and interleukin-1 [20, 21]. Studies have shown that exogenous use of IL-10 prevent IR due to fat accumulation [21, 22], and decreased serum levels of IL-10 have been associated with increased prevalence of metabolic syndrome and type 2 DM [23].

Although the role of afamin and IL-10 in IR and inflammation reduction has been studied separately, there is still limited and conflicting information about the role of PCOS on anti-inflammatory adipokines such as IL-10 and their mechanism of action. This study aimed to investigate the relationship between serum levels of afamin and IL-10 with IR in infertile women with PCOS.

Materials and methods

This prospective observational study was conducted on 88 patients admitted to Istanbul Medeniyet University Goztepe Training and Research Hospital Gynecology and Obstetrics Clinic and Private Medistate Kavacik Hospital Gynecology and Reproductive Medicine Clinic between December 2020 and

February 2021. Patients underwent routine IVF clinic examinations, and no special procedures were performed in the examination, treatment, and follow-up processes.

The study was performed according to the regulations established by the Clinical Research and Ethics Committee and the Helsinki Declaration of the World Medical Association. The study was conducted with the permission of the Research Ethics Committee of Istanbul Medeniyet University Goztepe Training and Research Hospital (Permission granted/CAAE number: 02.12.2020, Decision no: 2020/0703). All patients signed informed consents.

The criteria for inclusion in the study were consenting to participate in the study, being aged between 18 and 36 years, and having unsuccessfully attempted conceiving for at least one year. Those who disagreed to participate, those with DM, endocrinopathy, or hypertension, patients who smoked, or used drugs that altered the metabolism of insulin, lipids, and hormones up to three months before the study, patients with a deficiency of vitamins B6 and B12 and those taking vitamin supplements up to 6 months before the study were excluded.

Participants were divided into study and control groups based on PCOS diagnosis. The PCOS group consisted of women aged 18-36 years, who were referred to an infertility clinic during the study period and diagnosed with PCOS according to the 2003 Rotterdam Consensus criteria [24]. Women referred to our clinic for infertility during the study period and not diagnosed with PCOS were included in the control group. The PCOS and control group participants were divided into two groups based on IR, which was identified with The Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) index ≥ 2.5 . Sample size calculation with G*Power 3.1 and assessment of the mean of two groups with ANOVA test revealed that with an effect size of 35%, df of 4, power of 70% and 0.05 type 1 error, at least 16 patients were required in each group. Therefore, twenty-two patients were included in each group. The control groups were divided into two groups as those with and without IR so that the exact effect of IR could be measured in patients with PCOS and to minimize the potential bias. The final groups were as follows: 1) PCOS non-IR (HOMA-IR < 2.5), 2) PCOS+IR (HOMA-IR ≥ 2.5), 3) Control+non-IR (HOMA-IR < 2.5), 4) Control+IR (HOMA-IR ≥ 2.5).

Body Mass Index (kg/m^2) was calculated by measuring the height (m) and body weight (kg) obtained in the routine examination at the first visit. Waist circumference, hip circumference, and WHR were noted.

We examined the patients on the third day of their instrumentation after the examination. Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), Estradiol (E2), Prolactin, Thyroid Stimulating Hormone (TSH), Free T4, Anti-Mullerian Hormone (AMH), Hemogram, total cholesterol, LDL, HDL, Triglyceride, fasting blood glucose, fasting insulin, and HOMA-IR values were measured.

Serum samples were obtained by centrifuging venous blood samples for 10 minutes at 4000 rpm after a 30-minute coagulation period. Serum samples collected for biochemical and hormonal evaluation were analyzed in Medistate Kavacik Hospital's biochemistry and hormone laboratory. Afamin and IL-10 measurement were performed with Human AFM Antibody

Enzyme-Linked Immunosorbent (ELISA) and IL-10 ELISA Kits (Reader Biotek ELx800). The plates provided by the manufacturer were pre-coated with Human AFM/IL-10 antibody. AFM/IL-10 in the samples were bound to antibodies coated on the wells. The biotinylated Human AFM/IL-10 antibody was then added and bound to AFM/IL-10 in the sample. Streptavidin-HRP was added and bound to the Biotinylated AFM/IL-10 antibody. In between all steps and after the final incubation, the plates were washed. After final incubation, the substrate solution was added, which caused a color change proportional to the amount of Human AFM/IL-10 in the initial sample. The reaction was terminated by the addition of acid-stopping solution, and absorbance evaluation was made at 450 nm. Quantification was performed by calculation of concentrations from absorbance values according to the slope of the calibration curve obtained with the respective standards included in each ELISA kit.

Statistical analysis

Data were analyzed using SPSS for Windows, Version 23.0 (Armonk, NY: IBM Corp). A two-way ANOVA test was used to evaluate the significant difference between the studied variables in the PCOS/Control groups with different IR. The non-normal study variables were converted to normal by standardization, after which the appropriate test was used. The correlation of variables with Afamin and IL-10 was examined with the Pearson correlation test. For all tests, a *P*-value <0.05 was considered statistically significant.

Results

The mean age and BMI of all participants were 26.8 (5.1) years (range: 18-36 years) and 25.3 (4.3) kg/m² (range: 18.6 to 41.1 kg/m²), respectively. The BMI, WHR, AMH and LG values significantly differed between the groups, which were all highest in PCOS patients with IR (HOMA-IR ≥2.5) (*P*<0.001 for all).

As expected, fasting insulin was significantly higher in the two groups with IR (*P*=0.02). The Hematocrit and mean corpuscular volume (MCV) values significantly differed between the groups (*p*=0.02 and *p*=0.03, respectively), both being highest in the PCOS-IR group. The complete two-way ANOVA results for all groups are given in Table 1.

The results of Pearson correlation for Afamin and IL-10 variables are shown in Table 2. Afamin weakly positively correlated with BMI (*P*=0.005), and Hematocrit (*P*=0.001), moderately positively correlated with AMH (*P*<0.001), LH (*P*<0.001), and fasting insulin (*P*=0.001), weakly positively correlated with fasting blood glucose (*P*=0.02), and LDL (*P*=0.002), and weakly negatively correlated with leukocyte (*P*=0.003), and neutrophil count (*P*=0.002).

IL-10 was strongly negatively correlated with BMI (*P*<0.001), moderately negatively correlated with waist circumference (*P*<0.001), hip circumference (*P*<0.001), WHR (*P*<0.001), AMH (*P*<0.001), and LH (*P*<0.001), weakly negatively correlated with fasting insulin (*P*=0.001), MPV (*P*=0.04), and MCV (*P*=0.05), weakly positively correlated with monocyte count (*P*=0.03), and very weakly negatively correlated with hematocrit (*P*=0.05).

Table 1: Results of two-way ANOVA comparisons between the groups

	PCOS HOMA-IR <2.5	PCOS HOMA-IR ≥2.5	Controls HOMA-IR <2.5	Controls HOMA-IR ≥2.5	<i>P</i> - value
Afamin	178.7(46.6)	231.7(30.9)	75.9(17.6)	113.7(34.9)	0.2
IL-10	679.8(119.9)	181.4(52.09)	291.9(85.3)	487.5(116.9)	0.000
BMI	21.9(2.06)	30.2(4.3)	25.1(1.9)	23.7(2.4)	0.000
Waist circumference	75.5(9.2)	97(13.2)	87.9(12.8)	75.09(5.3)	0.000
Hip Circumference	95.7(6.1)	111.7(11.16)	105.5(11.0)	97.9(5.4)	0.000
WAIST HIP RATIO (WHR)	0.77(0.06)	0.8(0.1)	0.8(0.07)	0.7(0.05)	0.000
Age	26.5(5.3)	24.5(5.2)	27.2(5.04)	26(5.1)	0.3
AMH	1.5(0.3)	2.5(0.6)	1.6(0.5)	1.3(0.3)	0.000
FSH	6.04(1.8)	5.8(1.2)	5.5(1.6)	6.3(1.6)	0.1
LH	4.7(1.9)	9.8(4.04)	4.6(1.7)	5.3(1.8)	0.000
Estradiol	42.9(9.05)	43.5(19.7)	44.7(18.8)	44.1(20.3)	0.8
Free T4	1.09(0.2)	1.07(0.1)	1.6(2.1)	1.8(3.2)	0.8
TSH	2.02(0.7)	2(1.1)	1.8(1.02)	2.09(1.2)	0.6
Prolactin	15.8(5.5)	15.3(5.9)	16.8(5)	15.05(7.4)	0.6
Fasting blood glucose	91.7(8.1)	96.3(8.9)	85.8(5.4)	96.1(8.9)	0.09
Fasting insulin	6.4(1.9)	15.3(6.09)	7.4(1.5)	12.7(2.8)	0.02
Total Cholesterol	200.7(28.8)	188.2(40.3)	218.9(67.6)	192.8(50.5)	0.2
LDL	121.2(30.8)	124.5(31.3)	105.7(39.6)	93.3(35.7)	0.5
HDL	69.3(11.4)	60.8(15.2)	61.9(14.5)	64.5(24.8)	0.1
Triglyceride	107.7(31.2)	104.1(41.3)	114.1(67.8)	96.5(26.1)	0.4
Leukocyte	7.9(1.6)	7.8(1.8)	9.1(2.3)	9.4(2.3)	0.7
Neutrophil	4.6(1.5)	4.4(1.3)	5.6(1.6)	5.9(1.9)	0.4
Basophil	0.04(0.02)	0.1(0.3)	0.04(0.01)	0.06(0.02)	0.2
Lymphocyte	2.2(0.5)	2.3(0.7)	2.4(0.8)	2.4(0.7)	0.8
Monocyte	0.66(0.20)	0.5(0.2)	0.5(0.2)	0.590.1)	0.5
Hemoglobin	13.2(2.6)	13.7(0.7)	12.8(1.2)	12.9(1.1)	0.5
Hematocrit	38.4(2.9)	41.1(2.5)	37.8(3.3)	37.7(2.7)	0.02
Platelet	242636.3	272240.9	247409.1	271181.8	0.7
	(58359.70)	(38293.6)	(51100.8)	(40079.2)	
PCT	0.22(0.06)	0.24(0.03)	0.23(0.06)	0.23(0.1)	0.7
RDW	13.5(1.1)	12.5(1.3)	13.3(1.3)	12.8(0.8)	0.3
MPV	10.2(0.6)	9.5(0.7)	10.2(0.9)	9.9(0.7)	0.3
MCV	83.8(4.02)	86.4(3.2)	84.2(5.06)	82.8(5.3)	0.03

IL-10: Interleukin 10, BMI: Body mass index, AMH: Anti-Mullerian Hormone, FSH: Follicle stimulating hormone, LH: Luteinizing Hormone, TSH: Thyroid stimulating hormone, LDL: Low-density lipoprotein, HDL: High density lipoprotein, PCT: Procalcitonin, RDW: Red cell distribution width, MPV: Mean platelet volume, MCV: Mean corpuscular volume

Table 2: The Pearson correlation for Afamin and IL-10

	Afamin		IL10	
	Person Correlation	<i>P</i> -value	Person Correlation	<i>P</i> -value
Afamin				
IL-10	-0.08	0.4	-0.08	0.4
BMI	0.29	0.005	-0.67	0.000
Waist circumference	0.13	0.2	-0.57	0.000
Hip Circumference	0.1	0.3	-0.51	0.000
Waist Hip Ratio (WHR)	0.10	0.3	-0.47	0.000
Age	-0.02	0.8	-0.09	0.3
AMH	0.4	0.000	-0.47	0.000
FSH	0.03	0.7	0.16	0.1
LH	0.42	0.000	-0.4	0.000
Estradiol	-0.008	0.9	-0.14	0.18
Free T4	-0.06	0.5	-0.058	0.5
TSH	-0.04	0.7	0.05	0.5
Prolactin	-0.04	0.7	0.05	0.6
Fasting blood glucose	0.24	0.02	-0.05	0.6
Fasting insulin	0.35	0.001	-0.34	0.001
Total Cholesterol	-0.17	0.1	0.02	0.8
LDL	0.32	0.002	-0.002	0.9
HDL	0.09	0.4	0.13	0.2
Triglyceride	-0.07	0.4	-0.08	0.4
Leukocyte	-0.31	0.003	0.001	0.9
Neutrophil	-0.33	0.002	0.02	0.8
Basophil	0.12	0.2	-0.13	0.2
Lymphocyte	-0.11	0.2	-0.045	0.6
Monocyte	0.007	0.9	0.22	0.03
Hemoglobin	0.18	0.09	0.000	0.9
Hematocrit	0.33	0.001	-0.02	0.05
Platelet	0.02	0.8	-0.12	0.2
PCT	-0.03	0.7	-0.10	0.3
RDW	-0.066	0.5	0.16	0.1
MPV	-0.16	0.10	0.21	0.04
MCV	0.16	0.1	-0.2	0.05

IL-10: Interleukin 10, BMI: Body mass index, AMH: Anti-Mullerian Hormone, FSH: Follicle stimulating hormone, LH: Luteinizing Hormone, TSH: Thyroid stimulating hormone, LDL: Low-density lipoprotein, HDL: High density lipoprotein, PCT: Procalcitonin, RDW: Red cell distribution width, MPV: Mean platelet volume, MCV: Mean corpuscular volume

The different Afamin and IL-10 values in the four groups are presented in Figures 1 and 2, respectively.

Figure 1: Afamin values in the groups

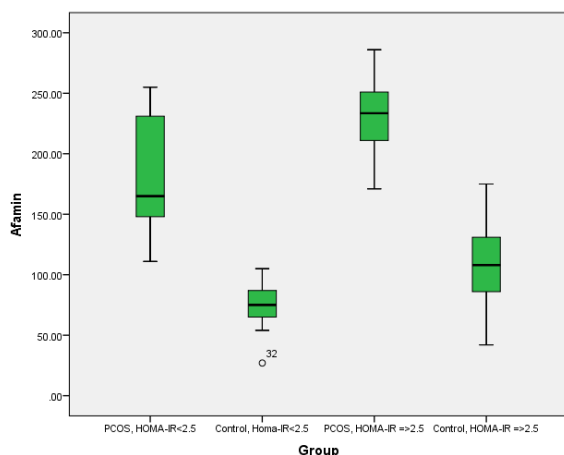
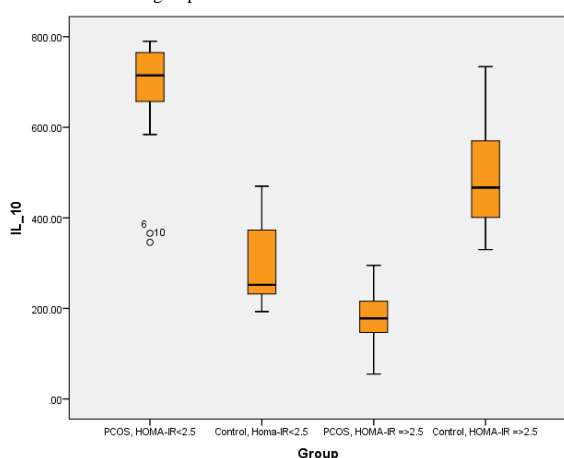


Figure 2: IL-10 values in the groups



Discussion

IR is an incomplete response of glucose to a certain amount of insulin [4]. In many of these patients, circulating insulin levels increase to neutralize this deficiency and maintain stable glucose levels. It is correlated with a wide range of manifestations, including cardiovascular disease, hypertension, type 2 DM, and lipid disorders. IR is prevalent in patients with PCOS, particularly in obese patients [2, 3]. However, the relationship between IR and variables such as afamin and IL-10 is unclear. Our results showed that afamin levels significantly increase in patients with PCOS compared to healthy groups and with increasing BMI. These results are consistent with Seeber et al. (25). In our study, afamin levels were significantly higher PCOS patients with IR compared to those without. Studies have shown that increased levels of afamin have been observed in patients with oxidative stress and inflammation [12-15]. A positive correlation between these two variables could be explained with the fact that inflammation and oxidative stress are common in PCOS patients with IR. In their study, Li et al. showed that afamin levels were significantly correlated with HOMA-IR in patients with PCOS. Because the authors found no correlation between increased afamin levels and BMI, they concluded that IR plays a major role in increasing PCOS patients' afamin levels [26].

Afamin level can be useful in IR-related tests. For example, afamin may be able to determine the need for an oral glucose tolerance test (OGTT), which is expensive and time-

consuming. However, since the replacement of OGTT-related biomarkers is beyond the scope of this study, these results can only be interpreted as preliminary findings.

Studies have shown that PCOS is associated with hyperinsulinemia and oxidative stress [27-29]. Since afamin is known as an E-binding protein [15], and vitamin E relates to the non-enzymatic antioxidants group, afamin may be a marker of vitamin E-related oxidative stress. Therefore, future studies are recommended to evaluate the oxidative stress status in PCOS patients and its relationship with afamin.

In this study, the results showed that the amount of IL-10 in the groups with IR was significantly lower than in those without IR. These results are consistent with previous findings [30-33]. IL-10 is an anti-inflammatory cytokine that is found less in overweight individuals [19, 20]. Our results also found a significant negative correlation between WHR and IL-10, indicating a decrease in IL-10 in overweight individuals. IR is one of the most common features of metabolic disorders such as diabetes and obesity [1, 2]. Although the relationship between IR and obesity is widely accepted, its mechanisms remain complex and controversial.

IR is common in patients with PCOS, especially obese patients [1-4]. Our study results showed that in patients with IR and obesity, the greatest decrease is observed in IL-10. These findings are consistent with the results of previous studies [20-23].

Obesity has a prevalence of more than 50% in PCOS patients [6]. Although obesity is not an integral or inherent complication of PCOS [7, 8], the development of IR and hyperinsulinemia due to overweight can contribute to the clinical complications of this syndrome [5]. About 50-70% of women with PCOS have some degree of IR [10], which may result from obesity or independently, and is associated with hyperandrogenism [13]. It has been suggested that IR can also play a leading role in the pathogenesis of this disease [9].

The present study observed that the mean HOMA-IR was significantly higher among PCOS patients compared to the healthy group, consistent with various other studies [34-37]. Contrarily, some studies showed this difference only in obese women with PCOS [38, 39]. This may be because of the effect of obesity on IR, independent of PCOS.

In this study, serum IL-10 levels were significantly lower in PCOS patients than in healthy women. Panidis et al. also observed a significant difference between serum IL-10 levels in PCOS patients and normal-weight healthy women (BMI < 25) [40]. Ardawi et al. reported that IL-10 was lower in women with PCOS than in healthy women of similar weight [41]. While there was a significant difference in fasting insulin between the groups, no significant difference was observed in terms of fasting glucose. These findings were consistent with the findings of Tarkun et al., which found higher fasting insulin and similar fasting glucose levels among women with PCOS [42].

Limitations

One of the limitations of this study is the small number of samples. It is recommended that further studies be performed with a larger number of patients to investigate the different variables and the independent effect of each on IR in PCOS patients. Lack of information about the distribution of fat in the women studied is another limitation of the present study.

Therefore, a more detailed study with more variables is required to investigate changes in IL-10 and afamin levels and evaluate the effect of central fat mass on it. Whether IR results from obesity or PCOS should also be considered more carefully.

Conclusion

In this study, the effect of afamin and IL-10 on IR in patients with PCOS was investigated. Our results showed that afamin levels in PCOS patients with IR increased significantly, which could be a sign of oxidative stress, while that of IL-10 levels decreased, which can be attributed to obesity. Further studies are needed to determine the relationship between IR, obesity, and PCOS.

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