Research Article

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Levels of integrin, irisin, and anti-Müllerian hormone in polycystic ovary syndrome patients

Polikistik over sendromlu hastalarda integrin, irisin ve anti-Müllerian hormon düzeyleri

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Abstract

Purpose: The purpose of the study was to find out if there were any notable differences in serum levels of irisin, integrin α4, integrin α5, integrin β2, and anti-Müllerian hormone (AMH) between PCOS patients and healthy women.

Material and methods: This case-controlled observational study includes 42 women with PCOS and 42 healthy women of the same age as the control group. Irisin, integrin α4, integrin α5, and integrin β2 levels were measured, along with serum lipid subfractions, fasting serum glucose, fasting insulin, and other hormones (androgens, gonadotropins). Insulin resistance was estimated using the Homeostasis Model Assessment (HOMA-IR). We measured the levels of irisin and integrin (α4, α5, and β2) using an enzyme-linked immunosorbent assay.

Results: Women with PCOS had significantly higher levels of irisin, HOMA-IR, AMH, and free androgen index (FAI) than the control group (*p*=0.0001, 0.0001, and 0.0001, respectively). Integrins (α4, α5, β2) are substantially reduced in women with PCOS (*p*=0.023, 0.001, and 0.0001, respectively). HOMA-IR scores showed a negative correlation with integrin levels (α4, α5, β2) (r=-0.412 *p*=0.007, r=-0.468 *p*=0.002, and r=-0.446 *p*=0.003, respectively) but a positive correlation with AMH and irisin levels in women with PCOS (r=0.693 *p*=0.001 and r=0.424 *p*=0.005, respectively).

Conclusion: AMH, irisin, and integrin levels were found to be significantly changed in non-diabetic women with PCOS. We think that the root cause of these changes could be disturbances in energy homeostasis.

Keywords: Integrin α4, integrin α5, integrin β2, irisin, polycystic ovary syndrome.

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Öz

Amaç: Çalışmanın amacı, PCOS hastaları ve sağlıklı kadınlar arasında serum irisin, integrin α4, integrin α5, integrin β2 ve anti-Müllerian hormonu (AMH) seviyelerinde belirgin bir fark olup olmadığını bulmaktı.

Gereç ve yöntem: Bu vaka kontrollü gözlemsel çalışmaya PCOS'lu 42 kadın ve kontrol grubuyla aynı yaştaki 42 sağlıklı kadın dahil edildi. İrisin, integrin α4, integrin α5 ve integrin β2 seviyeleri, serum lipid alt fraksiyonları, açlık serum glikozu, açlık insülin ve diğer hormonlar (androjenler, gonadotropinler) birlikte ölçüldü. İnsülin direnci, Homeostasis Model Assessment (HOMA-IR) kullanılarak hesaplandı. İrisin ve integrin (α4, α5 ve β2) seviyelerini enzim bağlantılı bir immünosorbent testi kullanarak ölçtük.

Bulgular: PCOS'lu kadınlarda irisin, HOMA-IR, AMH ve serbest androjen indeksi (FAI) düzeyleri kontrol grubuna göre önemli ölçüde daha yüksekti (*p*=0,0001, 0,0001, ve 0,0001, sırasıyla). İntegrinler (α4, α5, β2) PCOS'lu kadınlarda önemli ölçüde azalmıştır (*p*=0,023, 0,001, ve 0,0001, sırasıyla). HOMA-IR skorları integrin düzeyleriyle (α4, α5, β2) negatif korelasyon gösterirken (r=-0,412 *p*=0,007, r=-0,468 *p*=0,002, ve r=-0,446 *p*=0,003, sırasıyla) PCOS'lu kadınlarda AMH ve irisin düzeyleriyle pozitif korelasyon gösterdi (r=0,693 *p*=0,001 ve r=0,424 *p*=0,005, sırasıyla).

Sonuç: AMH, irisin ve integrin düzeylerinin PCOS'lu diyabetsiz kadınlarda önemli ölçüde değiştiği bulundu. Bu değişikliklerin temel nedeninin enerji homeostazındaki bozukluklar olabileceğini düşünüyoruz.

Anahtar kelimeler: İntegrin α4, integrin α5, integrin β2, irisin, polikistik over sendromu.

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Introduction

Among women of reproductive age, PCOS is the most prevalent endocrine condition. Depending on the diagnostic criteria applied, the prevalence of PCOS varies significantly; according to the Rotterdam criteria, it is approximately 18%, while the NIH/NICHD criteria are 4-8% [1]. PCOS, which has a complex endocrinological and metabolic presentation, is characterized by a range of symptoms brought on by androgenic status and ovarian dysfunction. Lifestyle and diet are two examples of the genetic and environmental factors that contribute to the complex multigenic structure of PCOS [2].

While obesity is prevalent among women with PCOS, not all of them are affected. PCOS symptoms are exacerbated by obesity, especially high visceral fat levels [3]. In PCOS patients, visceral fat also plays a role in insulin resistance (IR), which can result in diabetes mellitus and impaired glucose tolerance [3]. Thus, IR affects the pathophysiology of related disorders like metabolic syndrome and PCOS. Following binding, insulin receptor signaling is impaired in PCOS patients. Between 44 and 85 percent of women with PCOS have IR [3].

Women have follicles as large as 8 mm that produce anti-Müllerian hormone (AMH) [4]. AMH is not impacted by follicle-stimulating hormone (FSH) because it is secreted before FSH-dependent dominant follicle selection. AMH may also prevent premature maturation of developing follicles. Numerous investigations have found a relationship between the number of developing follicles and serum AMH levels. Additionally, as women age, their follicle count and serum levels of AMH decline [5]. But since AMH is only expressed by developing follicles, young women's serum concentration of the hormone is not correlated with the number of primordial follicles. Therefore, it is possible to think of AMH serum levels as a representation of functional ovarian reserve (FOR). FOR is composed of tiny, developing follicles that range in size from 2 to 5 mm. FSH chooses the dominant follicle for ovulation [5]. AMH levels have been observed in all PCOS populations and are two to four times higher in PCOS-afflicted women than in healthy women [6]. This increase is caused by an increase in the number of small follicles and secretion within them, and it is mainly seen in the preantral and small antral follicles [6].

According to some theories, irisin is crucial to the pathophysiology of PCOS, which is connected to IR and energy metabolism [7]. In addition to visceral and subcutaneous adipose tissues, skeletal muscle is the primary source of the adipokine irisin. Irisin increases glycogenesis while decreasing gluconeogenesis to increase insulin sensitivity. It is linked to nonalcoholic fatty liver disease, cardiovascular disease, obesity, lipid metabolism, PCOS, and type 2 diabetes [8]. Irisin uses integrins to carry out its function in certain tissues [8].

The extracellular matrix (ECM) is a complex structure made up of various proteins, proteoglycans, and polysaccharides that acts as a scaffold for cells and controls biological functions like adhesion, migration, repair, survival, and development. Integrins transmit signals through the plasma membrane to initiate intracellular signaling, which facilitates ECM cell adhesion and signaling into cells [9]. The underlying IR that causes type 2 diabetes may be treated with integrins and the extracellular matrix (ECM), which are significant insulin action regulators [9]. The α- and β-subunits that make up integrins are heterodimeric transmembrane receptors. They have the ability to form 24 distinct integrins, each with distinct ligand-binding and signaling characteristics [9].

Diabetes is known to be associated with PCOS. Additionally, type 2 diabetes and infertility are more likely to develop in later life in women with PCOS. We sought to investigate the possibility that AMH, irisin, and integrin (α4, α5, β2), which are primarily implicated in processes related to fertility and diabetes, could either directly or indirectly contribute to the development of PCOS. We analyzed serum levels of irisin, integrin α4, integrin α5, integrin β2, and AMH in PCOS patients versus healthy women in a control group. The study also examined how irisin, integrin (α4, α5, β2), and AMH levels correlate with IR.

Materials and methods

Study design and patients

Participants in the study were 137 Turkish women, ages 18 to 38, who had been admitted to the gynecology department of the study center on multiple occasions. Eight women who had pelvic pathologies, seven women who had systemic diseases, nine women who had acute infections, 10 women who regularly used drugs that affect or alter insulin secretion and function, sex hormones, and lipid profiles, 12 women who used alcohol and/or cigarettes, and seven women who refused to participate in the study were excluded. 42 women who satisfied the Rotterdam diagnostic criteria for PCOS were included in the study group, while 42 healthy women with regular menstrual cycles (2–7 days with 25–34 day periods) were included in the control group.

The diagnosis of PCOS required at least two of the following characteristics, per the Rotterdam diagnostic criteria [1]: irregular menstruation (cycle duration >35 or <21 days), biochemical (increased testosterone and free androgen index (FAI)) and/or clinical (modified Ferriman Gallwey score ≥ 5) hyperandrogenism, and polycystic ovarian morphology (PCOM; as determined by an ultrasound machine with a transvaginal probe of less than 8 MHz, at least one ovary has ≥12 follicles (diameters ranging from 2 to 9 mm) and/or an ovarian volume greater than 10 cm^3.

The study excluded participants with Cushing's syndrome, diabetes mellitus, endocrinopathy, including late-onset 21-hydroxylase deficiency, androgen-secreting tumors, taking drugs that contain progesterone and estrogen, infectious diseases, hypertension, hyperprolactinemia, thyroid dysfunction, chronic liver disease, sex hormone and lipid profile, or medications that affect or change insulin secretion and function.

Sample collection and determination of biochemical analyses

After a 12-hour fast, venous blood samples were taken from each study participant on days 3–5 of either progesterone-induced or spontaneous cycles. Triglycerides (TG), insulin, serum fasting glucose (FG), triglycerides (TG), total cholesterol (TC), HDL, sex hormone-binding globulin (SHBG), luteinizing hormone (LH), FSH, total testosterone, AMH, irisin, integrin α4, integrin α5, and integrin β2 were all measured. Low-density lipoprotein cholesterol (LDL) levels were determined using the Friedewald formula. FAI was calculated using the following formula: 100 X SHBG (in nmol/L) divided by total testosterone (in nmol/L) is FAI. The HOMA-IR score, which is computed as [fasting insulin concentration (mIU/L) x glucose (mmol/L) / 22.5], was used to identify IR.

Irisin levels were measured based on the sandwich ELISA method by using BT Lab products. This commercial kit's assay range is between 0.2 ng/ml – 60 ng/ml, and its sensitivity is 0.095 ng/ml. Irisin kit intra assay: CV<8%, inter assay: CV<10%.

Likely integrin subunits (α4, α5, β2) were measured by using BT Lab products. These subunits have sensitivity with 0.12 ng/ml, and their assay ranges are between 0.2 ng/ml – with the intra assay: CV<8% and inter assay: CV<10%.

Anti-mullerian hormon was measured on Cobas 801 hormon systems (Roche Cobas Systems, Manheim, Germany) by using electrochemiluminescence immunoassay. Limit of Blank (0.007 ng/mL), Limit of Detection (0.01 ng/mL), and Limit of Quantitation (0.030 ng/mL) were determined according to CLSI (Clinical and Laboratory Standards Institute) EP17 A2 requirements. For AMH, intra-assay CV was ≤8%. Total allowable error was ± 25%.

The blood samples were placed in a 10-cc venous blood biochemistry tube and centrifuged for 10 minutes at 3500 rpm after being allowed to stand at room temperature for approximately 15 minutes. After that, the serum samples were stored at -80°C until they could be analyzed. All kits and samples were allowed to come to room temperature before analysis. The samples were tested in accordance with the prospectus following the preparation of the chemicals and standards for the kits. A BioTek Elx800 Microplate reader (BioTek Instruments Inc., USA) was used to measure the absorbance values of the samples at 450 nanometers (nm). Using the Gen5 data analysis software, concentrations were calculated for serum absorbance values.

Anthropometric measurements

On the day that blood samples were collected, the same physician performed all anthropometric measurements. We took measurements of the subjects' weight, height, and waist circumference while they were barefoot and wearing light clothing. The distance between the top of the head and the bottom of the feet (shoeless) was used to measure height using a fixed stadiometer. On the day that blood samples were collected, the same physician performed all anthropometric measurements. We took measurements of the subjects' weight, height, and waist circumference while they were barefoot and wearing light clothing.

Sample size and statistical analysis

Each participant's age and BMI were matched at the beginning of the study. If the subjects were younger than two years old and had a BMI of less than 1 kg/m^2, they were matched with healthy controls in terms of age and BMI. A power analysis was performed before the study. According to the reference study results [10], they had a medium effect size (d=1.484). Assuming we can achieve a lower effect size (d=0.7), when at least 76 participants (at least 38 participants per group) were included in the study, that would result in 85% power with a 95% confidence level (5% type 1 error rate). SPSS v22.0 (Statistical Package for Social Sciences, Chicago, IL, USA) was used for all data analyses. A 95% CI and a *p*-value <0.05 were used to assess the data. The median (interquartile range) described non-normal data, and the mean (standard deviation) described continuous data with a normal distribution. Normality was evaluated using the Shapiro-Wilk test. Either the Mann-Whitney U test or the Student t-test revealed significant differences between the two groups. Group differences in categorical variables were evaluated using the chi-square test. To evaluate the relationships between the ordinal and continuous variables, the Spearman correlation coefficient was used to calculate the correlations.

Permission was obtained from the Pamukkale University Non-Interventional Clinical Research Ethics Committee for the study (date: 21.01.2020, and number: 02).

Results

Table 1 shows that there was no difference in age, waist circumference, BMI, FSH, or total testosterone levels between the PCOS and control groups (*p*=0.248, 0.508, 0.412, 0.130, and 0.110, respectively). Women with PCOS scored higher on hirsutism, FAI, LH, and the LH/FSH ratio than the control group (*p*=0.0001, 0.0001, 0.0001, and 0.0001, respectively). The control group had higher levels of SHBG (*p*=0.001).

Table 1. Clinical features and steroid levels for both the women with PCOS and the healthy controls

	Healty control (n=42)	Women with PCOS (n=42)	*p* value
Age, year	24.97±1.87	24.59±1.58	0.248 (z=-1.154)
Hirsutism Score	3.40±1.29	10.92±1.02	0.0001* (z=-7.96)
BMI, kg/m^2	24.07±1.02	23.90±0.85	0.412 (z=-0.82)
Waist, cm	80.19±2.51	79.57±2.69	0.508 (z=-0.663)
FSH, mIU/ml	6.36±1.30	5.89±1.62	0.13 (z=-1.513)
LH, mIU/ml	7.04±1.66	10.24±1.74	0.0001* (z=-6.533)
LH/FSH ratio	1.14±0.31	1.86±0.54	0.0001* (t=-7.358)
Total testosterone, ng/ml	0.32±0.03	0.33±0.04	0.110 (t=-1.617)
SHBG, nmol/l	36.32±5.54	21.89±1.56	0.0001* (z=-7.892)
FAI	4.55±0.94	7.67±0.98	0.0001* (z=-7.658)

PCOS: Polycystic ovary syndrome; kg: kilogram; m: meter; cm: centimeter; mIU: milli-International Unit; ml: milliliter; ng: nanogram
nmol: nanomole; L: Liter; BMI: Body Mass Index; FSH: Follicle-Stimulating Hormone; LH: Luteinizing Hormone
SHBG: Sex Hormone-Binding Globulin; FAI: Free Androgen Index. t: Independent samples t test; z: Mann Whitney U test
p values marked with * are <0.05

There was no significant difference between the groups' fasting glucose and HDL levels (*p*=0.265 and 0.064, respectively). Women with PCOS had significantly higher levels of triglycerides, LDL, fasting insulin, HOMA-IR, and total cholesterol (*p*=0.0001, 0.0001, 0.0001, 0.0001, and 0.0001, respectively). Women with PCOS had lower levels of integrins α4, α5, and β2 (*p*=0.023, 0.001, and 0.0001, respectively), but higher levels of AMH and irisin (*p*=0.0001 and 0.0001, respectively) (Table 2).

Table 2. Metabolic characteristics, AMH, irisin, integrin α4, integrin α5 and integrin β2 levels for both the women with PCOS and the healthy controls

	Healty control (n=42)	Women with PCOS (n=42)	*p* value
Fasting glucose, mg/dl	90.61±1.95	91.33±2.73	0.265 (z=-1.114)
Fasting Insulin, mIU/ml	8.89±1.14	13.34±1.50	0.0001* (z=-7.673)
HOMA-IR	1.99±0.25	3.01±0.38	0.0001* (z=-7.618)
Total cholesterol, mg/dl	152.69±4.21	172±4.38	0.0001* (z=-7.875)
Triglyceride, mg/dl	63.30±4.48	102.83±6.86	0.0001* (z=-7.898)
HDL, mg/dl	57.95±3.13	56.83±2.48	0.064 (z=-1.854)
LDL, mg/dl	79.19±3.35	95.59±3.54	0.0001* (z=-7.907)
AMH, ng/ml	3.62±1.86	6.77±2.76	0.0001* (z=-4.829)
Irisin, ng/ml	21.42±7.57	26.78±6.81	0.0001* (z=-3.642)
Integrin α4, ng/ml	16.99±4.94	14.57±4.32	0.023* (z=-2.273)
Integrin α5, ng/ml	19.42±4.58	16.59±3.90	0.001* (z=-3.36)
Integrin β2, ng/ml	473.57±64.51	430.48±60.73	0.0001* (z=-3.883)

PCOS: Polycystic ovary syndrome; HOMA-IR: homeostasis model assessment; HDL: high-density lipoprotein-cholesterol
LDL: low-density lipoprotein-cholesterol; AMH: anti-Müllerian hormone; mg: milligram; dl: deciliter; mIU: milli-International Unit
ng: nanogram; ml: milliliter. z: Mann Whitney U test; *p* values marked with * are <0.05
HOMA-IR was calculated by converting fasting glucose values to nmol/L

In PCOS women, HOMA-IR scores had a negative correlation with integrin α4 (r=-0.412, *p*=0.007), integrin α5 (r=-0.468, *p*=0.002), and integrin β2 levels (r=-0.446, *p*=0.003), but a positive correlation with AMH (r=0.693, *p*=0.000) and irisin levels (r=0.424, *p*=0.005) (Table 3).

AMH levels were significantly positively correlated with LH levels (r=0.445 *p*=0.003) and FAI (r=0.496, *p*=0.001) in women with PCOS (Table 4).

Table 3. Correlation between HOMA-IR and AMH, irisin, integrin α4, integrin α5 and integrin β2 levels in women with PCOS

		AMH (ng/ml)	Irisin (ng/ml)	Integrin α4 (ng/ml)	Integrin α5 (ng/ml)	Integrin β2 (ng/ml)
HOMA-IR	r	0.693	0.424	-0.412	-0.468	-0.446
	p	0.001*	0.005*	0.007*	0.002*	0.003*

Spearman's correlation analyses were used to discover the association between variables, *p* values marked with * and in italics are <0.05
HOMA-IR: homeostasis model assessment, AMH: anti-Müllerian hormone, ng: nanogram; ml: milliliter

Table 4. Correlation between AMH and LH and FAI levels in women with PCOS

		LH (mIU/ml)	FAI
Serum AMH level, ng/ml	r	0.445	0.496
	p	0.003**	0.001**

Spearman's correlation analyses were used to discover the association between variables, *p* values marked with * and in italics are <0.05
AMH: anti-Müllerian hormone; LH: Luteinizing Hormone; FAI: Free Androgen Index, mIU: milli-International Unit; ml: milliliter

Discussion

This study aimed to investigate the possibility that AMH, irisin, and integrin (α4, α5, β2), which are mainly implicated in processes related to fertility and diabetes, could also play a direct or indirect role in the development of PCOS. The PCOS group showed significantly higher levels of AMH and irisin but significantly lower levels of integrin α4, α5, and integrin β2 compared to the control group. In women with PCOS, we discovered strong negative correlations between HOMA-IR scores and integrins α4, α5, and β2, but a significant positive correlation between HOMA-IR scores and AMH and irisin levels.

There were no differences in waist circumference, BMI, or age between the study's groups. This result suggests that the comparative analyses were consistent and that the groups shared comparable clinical features.

By inhibiting hepatic glucose synthesis and promoting glucose uptake by insulin-sensitive tissues such as skeletal muscle, liver, heart tissue, and adipose tissue, insulin controls glucose homeostasis. When pancreatic function is normal, insulin resistance (IR) is the result of insulin's reduced capacity to perform the metabolic processes necessary for glucose uptake, production, and lipolysis. This leads to compensatory elevated insulin levels both before and after glucose loading. Regardless of BMI, the precise mechanism of IR in PCOS remains unclear. 50–80% of PCOS women had IR, according to Wang et al. [11].

We found that, independent of BMI, women with PCOS had higher HOMA-IR and fasting insulin values. The idea that IR may start in PCOS patients before obesity develops is supported by these findings. Researchers have also discovered that PCOS patients had higher fasting insulin and HOMA-IR levels than control subjects [12]. It was only recently that the mechanisms through which IR works were introduced [13]. Through the accumulation of non-esterified fatty acids, IR promotes lipolysis in the skeletal muscles and liver muscles. Intrahepatic lipid accumulation affects insulin signaling and gluconeogenesis by inhibiting the insulin receptor and activating the diacylglycerol/protein kinase C axis. By modifying GLUT-4 expression and glucose uptake, blocking phosphoinositide-3 kinase, and phosphorylating insulin receptor substrate 1 in skeletal muscle, insulin signaling is reduced [13].

The ovaries and other non-insulin-sensitive tissues are overstimulated due to compensatory hyperinsulinemia for IR. In theca cells, insulin and LH in particular cooperate to promote the production of ovarian androgen. Additionally, by promoting the expression of LH, insulin, and IGF receptors in granulosa cells and indirectly by interfering with the regulation of the hypothalamic-pituitary-ovarian axis, insulin acts as a co-gonadotropin, raising LH activity. Hyperinsulinemia increases the adrenal steroid response to ACTH stimuli and inhibits the liver's synthesis of SHBG, which raises total and free androgen levels. According to this study, women with PCOS had greater levels of triglycerides, total cholesterol, LDL, FAI, LH, and the LH/FSH ratio than the control group. The hypothesis that IR triggers the development of hyperandrogenism in PCOS patients and that these effects have direct and/or indirect synergistic effects on one another may be the main cause of the chronic process and many of the symptoms of PCOS. These findings are consistent with this theory.

Initially, it was believed that the only reason for the increase in AMH concentrations in PCOS women was the presence of more preantral and small antral follicles. In vitro, normal-ovulatory PCOS patients produced 20 times more AMH than healthy controls, while anovulatory PCOS patients produced 75 times more [6].

Although the exact cause of this excess AMH production is unknown, there is evidence that androgens may be to blame. Serum androgen levels were found to positively correlate with AMH concentrations [14]. AMH receptor type II has been linked to intrinsic dysregulation of granulosa cells, which is another cause of this [14]. Excess AMH is thought to play a significant role in the distinctive follicular arrest of PCOS because it disrupts the expression of aromatase and the function of FSH [14].

In addition to directly stimulating AMH in small antral follicles, FSH may also increase E2 production in larger follicles, which could suppress AMH expression through the negative feedback of E2 [15]. LH also stimulates the production of AMH, according to clinical studies [15]. Compared to women who ovulate normally, PCOS women exhibit higher levels of luteinizing hormone (LH) receptor expression in both theca and granulosa cells of small antral follicles [16]. High levels of LH are combined with this in PCOS, resulting in premature luteinization of granulosa cells and hyperstimulation of theca cells. LH levels and AMH concentrations are positively connected in PCOS patients, and elevated LH levels cause theca cells to produce more ovarian androgen. This implies that AMH, GnRH, and LH have a positive feedback loop in PCOS [5]. Our study found that women with PCOS had higher serum AMH levels. We also discovered a positive correlation between FAI, LH, and AMH levels, which is consistent with earlier studies. Our results imply that a number of factors, such as increased preantral follicle count and elevated LH and androgen levels, may contribute to AMH elevation in PCOS patients.

It has been demonstrated that the exercise-induced cytokine irisin has a number of possible beneficial effects on insulin sensitivity and glucose homeostasis, including lowering adipogenesis, gluconeogenesis, and lipid accumulation and raising energy expenditure, glucose uptake, and glycogenolysis [17]. PCOS's progression is significantly influenced by IR, despite the fact that its aetiology is unknown. The connection between irisin and IR is now a fascinating but contentious subject as a result. In women with PCOS, circulating irisin levels and IR have a positive correlation, per Foda et al. [18] and Li et al. [7]. Researchers looked at the connection between irisin and insulin to ascertain irisin's function in IR. Insulin levels have been demonstrated to be negatively correlated with irisin in both healthy individuals and individuals with early-stage diabetes [19]. This relationship turns positive, though, as insulin sensitivity decreases; for instance, it has been demonstrated that individuals with diabetes have higher levels of irisin [20]. According to our research, irisin levels vary depending on whether PCOS is present, with women with PCOS having higher irisin levels. According to Wang et al. [11], irisin levels and IR in PCOS patients were unrelated. Irisin levels, FAI, IR, and hyperandrogenism were all markedly elevated in obese women with PCOS, according to another study [21]. Irisin levels and HOMA-IR scores in our study showed a strong correlation, with irisin levels rising or falling in direct proportion to changes in HOMA-IR scores. Choi [22] and Liu [23] found no significant correlation between irisin levels and IR, while Mareno et al. [19] reported a negative relationship.

Overall, the research's heterogeneity can be blamed for the conflicting results about irisin in PCOS. On the other hand, this might suggest that the body has experienced a decompensation period. In order to preserve metabolic balance, PCOS patients may secrete more irisin due to their underlying endocrine condition. According to our research, PCOS patients may have higher irisin levels as a result of compensating for IR and its side effects. More details regarding the clinical stages of the disease would be revealed by irisin level cut-off values in more sophisticated studies with bigger sample sizes.

Six integrin α subunits (α1, α2, α3, α4, α5, and α6), all linked to the β1 integrin subunit, are expressed in the liver [24], whereas seven integrin α subunits (α1, α3, α4, α5, α6, α7, and αv) are expressed in skeletal muscle [25]. Since integrins don't have kinase activity, downstream signaling is facilitated by the focal adhesion kinase (FAK) and integrin-linked kinase (ILK). FAK is a tyrosine kinase that regulates intracellular signaling, cytoskeleton stabilization, and focal adhesion turnover. It is regulated by the fibroblast growth factor receptor (FGFR), the insulin receptor (IRc), and the epidermal growth factor receptor (EGFR). FAK signaling controls adipocyte survival, which in turn controls insulin sensitivity in adipose tissue [26].

In addition to other cytoskeleton-associated proteins, ILK interacts with the cytoplasmic domains of β1, β2, and β3-integrin. There may be an interaction between insulin signaling and the extracellular matrix. Mice with striated muscle-specific integrin β1 deficiency have decreased AKT Ser-473 phosphorylation, which hinders insulin-stimulated skeletal muscle glucose uptake and glycogen synthesis [27]. Integrins control the metabolism of glucose in muscles by affecting vascularization and the transport of glucose through Glut4. Additionally, in mice with diet-induced insulin resistance, it has been suggested that ILK controls muscle capillarization [28]. Since the integrin-signaling molecule results in hyperglycemia and hyperinsulinemia in adult mice with general ILK depletion, as well as downregulation of GLUT4 expression, decreased insulin sensitivity, and Ser473 AKT phosphorylation, it is hypothesized that ILK may be a molecular target and prognostic biomarker of IR [29].

In the immune system, integrins like β2 are vital because they are involved in leukocyte trafficking and function. Integrin β2 is regulated by talin and kindlin-3 as cytoplasmic intracellular domains, which exacerbates the IR state by increasing neutrophil production and infiltration into muscle. Integrin β2 affects skeletal muscle and adipose tissue to control glucose homeostasis during a high-fat diet (HFD) [30]. According to our research, integrin α4, α5, and integrin β2 levels were considerably lower in PCOS-afflicted women than in the control group. We discovered a significant negative correlation between integrin α4, α5, and integrin β2 levels and HOMA-IR scores in women with PCOS. The hypothesis that a reduction in integrin levels in PCOS patients may be a major factor in the development of IR independent of obesity is supported by these findings in conjunction with the previously mentioned literature.

A noteworthy finding of this study was the negative correlation between integrin β2 levels and insulin resistance, which, to our knowledge, has not been previously reported. This may suggest a compensatory mechanism or a regulatory role of integrins in glucose metabolism that warrants further investigation.

As a result, a decrease in integrin levels could serve as the starting point for all laboratory and clinical changes in PCOS. However, more research is needed to determine whether this decrease is due to genetic disorders, excessive consumption, or another cause, such as malnutrition.

The current study has the following limitations: (1) it is a single-center study; (2) other integrins and enzymes, such as FAK and ILK, were not studied due to budget constraints; and (3) no group with DM and PCOS coexistence was formed. Moreover, the cross-sectional design limits the ability to draw conclusions about causality.

In conclusion, women who have PCOS are more likely to develop T2D later in life. The current study found that AMH, irisin, and integrin levels were significantly altered in non-diabetic women with PCOS. We believe that disruptions in energy homeostasis are the primary cause of these changes, but that these changes may also increase the chronicity and severity of PCOS, creating a vicious cycle. More research is needed to clarify this situation.

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