Bone mineral density in relation to polycystic ovary syndrome: an insight into irisin and insulin

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Background and aim Polycystic ovary syndrome (PCOS) is a complex metabolic and endocrine disorder. The influence of different metabolic and endocrine changes in women with PCOS and their relevance to bone status remains to be documented. Irisin is a newly identified adipo-myokine, which may play a role in the etiopathogenesis of PCOS as well as bone metabolism.
The aim of the study was to assess bone mineral density (BMD) and serum irisin level in women with PCOS and to determine BMD relationship with irisin and other hormonal parameters.

Patients and methods The study enrolled 80 women of reproductive age having PCOS and 15 age-matched and BMI-matched healthy women to serve as controls. A metabolic panel, reproductive hormones, and serum irisin level were measured. In addition, BMD of the spine and femur was also assessed using dual-energy X-ray absorptiometry.

Results Serum irisin level, fasting insulin, and homeostatic model assessment of insulin resistance were significantly higher in the PCOS group compared with the control group. Receiver operating characteristic curve for serum irisin was done for the PCOS group and the control group and demonstrated that the cut-off value for serum irisin was 0.161 μg /dl. There was also a statistically significant difference between the PCOS group and the control group in BMD of spine and femur, being higher in the PCOS group. Logistic regression analysis has shown that serum irisin level, waist circumference, and fasting serum insulin were predictors for the *z*-score of spine in the PCOS group.

Conclusion Serum irisin level may be considered as a novel biomarker for PCOS diagnosis. Circulating irisin in PCOS is strongly related to BMD. This suggests that irisin as an adipo-myokine may also be associated with bone metabolism.
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Introduction

Polycystic ovary syndrome (PCOS) is one of the most common endocrinopathies among women during the reproductive age, which is often characterized by obesity, hyperandrogenism, infertility, and dysfunctional uterine bleeding [1]. It is also associated with insulin resistance (IR), glucose intolerance [2], an augmented risk of metabolic syndrome (MS), and diabetes mellitus (DM) [3].

Irisin, a newly identified skeletal muscle hormone, has been shown to be processed from the product of fibronectin type III domain containing 5 (FNDC5) [4]. It stimulates browning of the white adipose tissue (WAT). This thermogenic program is accompanied by increased mitochondrial density and rate of oxygen consumption, inducing heat and weight loss [5].

Irisin may play a part in the pathophysiology of some diseases characterized by insulin resistance like MS and type 2 DM [5] and in the etiopathogenesis of PCOS [6]. Irisin was also reported to stimulate osteoblast differentiation *in vitro* [7].

PCOS might have an impact on bone metabolism [8]. As strong data on bone mineral density (BMD) and fracture risk in PCOS are lacking, we aim in this study to compare BMD and serum irisin level between the PCOS and control women and to investigate any association of BMD with serum irisin level and further hormonal parameters in women having PCOS.

Patients and methods

Study population

The study was conducted during the period from December 2016 to August 2017. A total of 95 women in their reproductive age were enrolled in this case–control study. Eighty women diagnosed as PCOS, in the age range of 22–40 years, were recruited from the gynecology, obstetric, and endocrinology outpatient clinics of Al-Zahra and Al-Hussein University Hospitals in Cairo. The control group included 15 healthy regularly menstruating, fertile women who were age and BMI matched with the

case group. They had no clinical or biochemical manifestations of hyperandrogenism. They were purposively selected from the employees at Al-Zahra University Hospital.

The patients included in this study were diagnosed as PCOS cases according to the definition of the new Rotterdam criteria (2003) when the patients had two of the following three features [9]:

(1) Menstrual cycle disturbances such as oligomenorrhea and amenorrhea.
(2) Polycystic ovaries on ultrasonography.
(3) Clinical and/or biochemical features of hyperandrogenism.

Exclusion criteria for all cases included age less than 22 years or greater than 40 years, a history of endocrine disorders that could alter metabolic and hormonal profile, systemic disease that could alter insulin sensitivity such as type 2 diabetes mellitus (T2DM).

Women on medications for greater than or equal to 3 months prior to the study (including oral contraceptives, glucocorticoids, ovulation induction agents, and estrogenic or antiandrogenic drugs or any medication for dyslipidemia or antiobesity drugs that could alter the patient's clinical presentation or hormonal profile) were excluded.

Ethical issues

All patients and controls were informed about the aim of the study and accepted the diagnostic procedure. Their participation in this study was fully voluntary and a verbal consent was taken from all patients included in the study.

Methodology

All participants were subjected to the following:

(1) Complete medical history was taken including age, menstrual history, fertility, parity, hirsutism, acne, and family history of T2DM.
(2) Complete clinical examination: including anthropometric parameters, such as weight, height, waist circumference (WC), and BMI calculation. Blood pressure was also measured. BMI is calculated as follows:

    BMI=weight (kg)/height^2 (m^2) [10].

Patients were checked for signs of hyperandrogenism and were assessed for hirstutism.

For patients who had hirsutism, the degree was evaluated by using a modified Ferriman–Gallwey score (mFG) [11]. The mFG defines hirsutism over 9 body parts and an mFG score greater than or equal to 8 defines hirsutism.

Laboratory investigations

Studies were performed during the follicular phase, between days 5 and 10 of the menstrual cycles, or in amenorrhea after excluding gestation. Samples were obtained after an overnight fast between 8:00 and 9:00 h

The following hormonal profile was assessed for patients: serum follicular stimulating hormone (FSH) and luteinizing hormone (LH).

FSH reference range [12] is as given below:

(1) Follicular phase 3.5–12.5 mU/ml.
(2) Ovulatory phase 4.7–21.5 mU/ml.
(3) Luteal phase 1.7–7.7 mU/ml.

LH reference range [13] is as given below:

(1) Follicular phase 2.4–12.6 mU/ml.
(2) Ovulatory phase 14–96 mU/ml.
(3) Luteal phase 1.0–11.4 mU/ml.
(4) Serum estradiol (E2) and prolactin. Estradiol reference range [12]: follicular phase 12.4 (6.2–16.7) pg/ml.
(5) Ovulation phase 41 (28.6–43.2) pg/ml.
(6) Luteal phase 22.3 (7.69–34.6) pg/ml.

The following biochemical profile was done for patients and controls:

(1) Serum total testosterone and sex hormone binding globulin (SHBG). Testosterone reference range: 0.06–0.82 ng/ml [14]. SHBG reference range (17–50 years) 26.1–110 nmol/l [13].
(2) Calculation of free androgen index (FAI).
(3) FAI=(total testosterone/SHBG)×100, with both TT and SHBG expressed in nanomoles per liter [15].
(4) Lipid profile including serum total cholesterol, triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C).
(5) Serum thyroid stimulating hormone (TSH) and free thyroxin (FT4). TSH reference range of 0.27–4.2 μIU/ml and freeT4 reference range: 1.0–1.6 ng/dl [16].
(6) Fasting serum insulin and fasting plasma glucose, insulin reference range: 2.6–24.9 mU/ml [17].

(7) Calculation of homeostatic model assessment of insulin resistance (HOMA-IR).
(8) It is an index used for the assessment of IR. It was measured by using the following equation [18]:
(9) HOMA-IR=[glucose (mg/dl)×insulin (IU/ml)]/405.
(10) Total serum calcium and phosphorus.
(11) Serum irisin level.

Blood collection

Three milliliter of 8 h fasting venous blood samples was withdrawn from each woman in the study and other samples were withdrawn for lipid profile assessments after 12 h fast.

Samples were allowed to clot for 2 h at room temperature before centrifugation for 15 min at 1000*g*. Serum samples were removed and FBG analysis was assayed immediately and the rest of the serum samples were stored at −20°C or −80°C for the determination of calcium, phosphorous, hormones, and irisin. Determination of fasting blood glucose, calcium, phosphorous, and lipid profile was carried out on Cobas e411 analyzer by colorimetric techniques.

Hormones were determined on Cobas e 411 analyzer (Roche Diagnostics International Ltd, Rotkreuz, Switzerland) using electrochemiluminescence immunoassays.

Irisin was determined using competitive enzyme immunoassay supplied by Phoenix Pharmaceuticals Inc. Burlingame, California, USA) [19].

Assessment of bone mineral density

Determination of BMD on lumber spine and left femur using dual-energy X-ray absorptiometry scan. This was done using MEDIX DR dual-energy X-ray absorptiometry scan.

Statistical analysis

The quantitative data were presented as mean, standard deviations, and ranges when their distribution was found parametric while qualitative data were presented as number and percentages.

The comparison between two independent groups with qualitative data was done by using χ^2-test and/or Fisher's exact test only when the expected count in any cell was found to be less than 5.

The comparison between two independent groups with quantitative data and parametric distribution was done by using independent *t*-test. Pearson's correlation coefficients were used to assess the correlation between two quantitative parameters in the same group. Linear regression analysis was used to assess the predictors of irisin and BMD parameters. The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the *P* value was considered significant if *P* less than 0.05. Statistical analysis was performed with the statistical package for social sciences (IBM SPSS, statistics for windows, version 23.0 ; Armonk ,NY ,United States of America; IBM Corp) version 23.

Results

The clinical characteristics of both groups: PCOS and control groups are presented in Table 1. There was no statistically significant difference between PCOS and control groups regarding age, anthropometric measurements, BP, and obesity prevalence.

No statistically significant difference was found between PCOS and control groups regarding fasting plasma glucose, serum calcium, serum phosphorus, and all parameters of lipid profile. However, fasting serum insulin, HOMA-IR, and serum irisin levels were significantly higher in the PCOS group compared with the control group ($P<0.05$) (Table 1, Figs 1 and 2).

There was no statistically significant difference regarding serum total testosterone, serum SHBG, and FAI (Table 1).

Z-score of spine and femur were significantly higher in the PCOS group compared with the control group ($P<0.05$) (Table 1).

A statistically significant positive correlation was found between *z*-score of both spine and femur and the anthropometric parameters including weight ($r=0.286$, $P=0.01$; $r=0.399$, $P=0.00$), BMI ($r=0.258$, $P=0.021$; $r=0.42$, $P=0.00$) and WC ($r=0.347$, $P=0.002$; $r=0.423$, $P=0.00$), respectively, in the PCOS group (Table 2).

No significant correlation was found between the *z*-score of both spine and femur and biochemical profile among the PCOS group (Table 2).

No significant correlation was found between the *z*-score of both spine and femur and hormonal profile (regarding FSH, LH, prolactin, E_2, TT, and FT_4) among the PCOS group (Table 2).

Table 1 Comparison between the polycystic ovary syndrome group and the control group in all studied parameters

	PCOS group (*N*=80)	Control group (*N*=15)	Test value*	*P* value	Significance
Age					
Mean±SD	27.55±3.73	27.87±5.66	−0.276	0.783	NS
Range	22–39	24–40			
Height (cm)					
Mean±SD	158.96±12.43	160.67±4.15	−0.524	0.602	NS
Range	161–176	153–168			
Weight (cm)					
Mean±SD	77.00±17.23	72.20±8.66	1.051	0.296	NS
Range	50–155	62–95			
BMI					
Mean±SD	29.44±5.21	28.01±3.65	1.020	0.311	NS
Range	19.7–45.7	23.8–34.8			
WC (cm)					
Mean±SD	83.56±9.77	82.93±7.37	0.237	0.813	NS
Range	58–121	70–96			
SBP (mmHg)					
Mean±SD	115.13±9.14	111.33±9.15	−1.474	0.144	NS
Range	100–130	100–130			
DBP (mmHg)					
Mean±SD	76.63±8.56	76.67±9.00	0.017	0.986	NS
Range	60–90	60–90			
Obesity prevalence [*n* (%)]					
Normal	6 (7.5)	1 (6.7)	2.272	0.518	NS
Over weight	37 (46.2)	10 (66.7)			
Obese	36 (45.0)	4 (26.7)			
Morbid obese	1 (1.2)	0 (0.0)			
FPG (mg/dl)					
Mean±SD	86.99±6.15	88.40±6.08	−0.817	0.416	NS
Range	75–98	77–96			
Ca (mg/dl)					
Mean±SD	9.32±0.37	9.47±0.43	−1.410	0.162	NS
Range	8.6–10.2	8.8–10.2			
Phosphorus (mg/dl)					
Mean±SD	3.73±0.39	3.51±0.44	1.914	0.059	NS
Range	3–4.8	2.9–4.2			
HOMA-IR					
Mean±SD	7.01±2.05	2.60±0.68	6.730	0.000	S
Range	3.9–12.13	1.64–3.67			
Cholesterol (mg/dl)					
Mean±SD	192.78±36.08	172.60±46.77	1.893	0.061	NS
Range	123–293	101–254			
TG (mg/dl)					
Mean±SD	145.20±52.30	127.80±82.96	1.067	0.289	NS
Range	65–354	55–396			
HDL-C (mg/dl)					
Mean±SD	47.81±12.02	49.07±9.07	−0.386	0.701	NS
Range	30–83.8	34–65			
LDL-C (mg/dl)					
Mean±SD	129.47±42.97	120.00±18.57	0.836	0.405	NS
Range	39–412	99–168			
TG/HDL ratio					
Mean±SD	3.23±1.46	2.56±1.49	−1.622	0.108	NS
Range	0.84–8.14	1.31–7.61			
Insulin (IU/ml)					
Mean±SD	32.52±8.74	11.80±2.70	7.424	0.000	S
Range	20–55.2	7.8–15.5			
Irisin (μg/dl)					

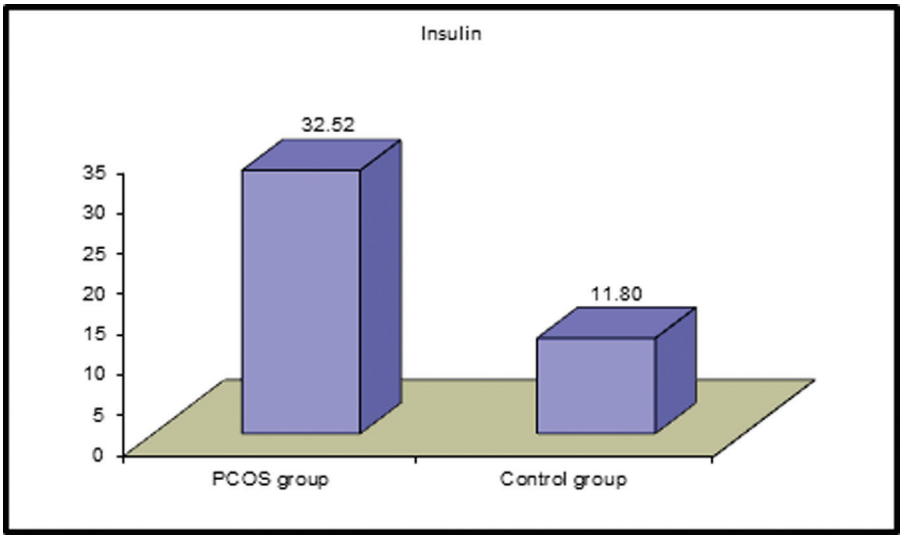
(*Continued*)

Table 1 (*Continued*)

	PCOS group (*N*=80)	Control group (*N*=15)	Test value*	*P* value	Significance
Mean±SD	0.41±0.16	0.14±0.01	5.441	0.000	S
Range	0.17–0.68	0.13–0.16			
TT (nmol/l)					
Mean±SD	0.44±0.60	0.24±0.13	1.290	0.200	NS
Range	0.08–3.58	0–0.51			
SHBG (nmol/l)					
Mean±SD	166.82±55.75	169.79±74.83	–0.179	0.858	NS
Range	41.4-231.16	18.8–223			
FAI					
Mean±SD	0.33±0.47	0.26±0.30	0.522	0.603	NS
Range	0.04–2.67	0.06–0.96			
Spine (*z*-score)					
Mean±SD	0.02±1.35	–0.77±1.41	2.046	0.044	S
Range	–2.8 to 5.6	–2.8 to 2.8			
Femur (*z*-score)					
Mean±SD	0.43±1.05	–0.74±0.96	4.002	0.000	S
Range	–2.5 to 3.6	–2.3 to 0.7			

Ca, calcium; DBP, diastolic blood pressure; FAI, free androgen index; FPG, fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance; LDL-C, low-density lipoprotein cholesterol; MS, metabolic syndrome; S, significance; SBP, systolic blood pressure; SHBG, sex hormone binding globulin; TG, triglyceride; TT, total testosterone; WC, waist circumference. *Independent *t*-test. *P*>0.05, nonsignificant. *P*<0.05, significant.

Figure 1



Fasting insulin level in the polycystic ovary syndrome and control groups.

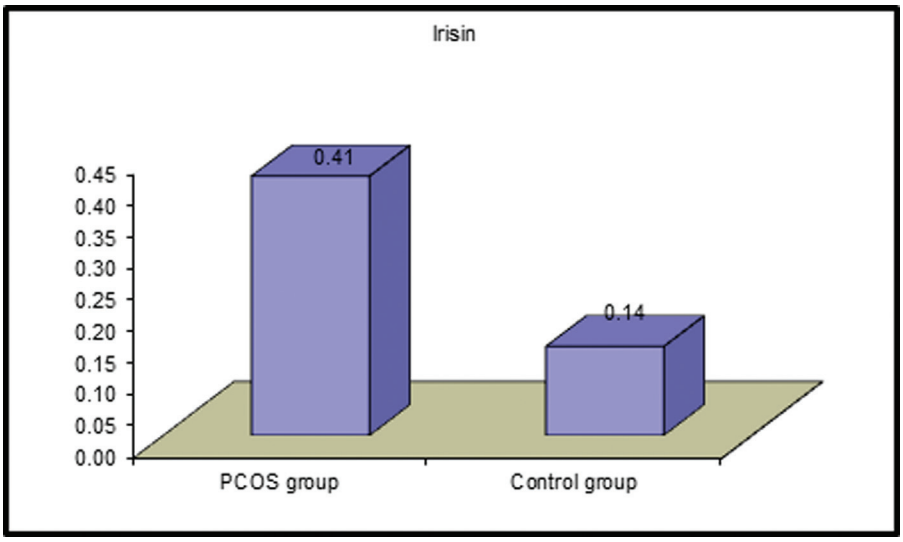
Z-score of the femur was negatively correlated with TSH (*r*=-0.221, *P*=0.049) and SHBG (*r*=-0.0225, *P*=0.045), and positively correlated with FAI (*r*=0.24, *P*=0.032) and HOMA-IR (*r*=0.267, *P*=0.017). However no correlation was found between the *z*-score of spine and TSH, SHBG, FAI, and HOMA-IR (Table 2, Figs 3 and 4).

A statistically significant positive correlation was found between the *z*-score of both spine and femur and fasting insulin (*r*=0.251, *P*=0.025; *r*=0.307, *P*=0.006, respectively) and serum irisin level (*r*=0.249, *P*=0.026; *r*=0.305, *P*=0.006, respectively) in the PCOS group (Table 2, Figs 5–8).

Receiver operating characteristic curve for serum irisin level of the study population has shown that the cut-off value for irisin level for the PCOS group and the control group was 0.161 µl/dl with a sensitivity of 100% and specificity of 100% (Table 3 and Fig. 9).

Linear regression analysis revealed that serum irisin level is the most significant predictor for *z*-score of spine in PCOS group then WC and fasting serum insulin, respectively (Table 4).

Figure 2



Serum irisin level in the polycystic ovary syndrome and control groups.

Discussion

In the present study, the prevalence of MS was significantly higher in the PCOS group compared with the control group (26.2 vs. 0% respectively, *P*=0.025).

Sobti *et al.* [20] have found that the prevalence of MS among the PCOS group was 36%.

Pikee *et al.* [21] found that the prevalence of MS was 36.02% in patients having PCOS and 10% in controls.

Hyperinsulinemia and IR are thought to be the key pathological factors for PCOS. The gold standard for establishing IR is euglycemic hyperinsulinemic clamp. However, this elaborate procedure is not suitable as a screening method. In the present study, IR assessment had been based on HOMA-IR values.

In the present study, fasting insulin and HOMA-IR were significantly higher in PCOS cases compared with the controls (*P*=0.000). These results are in agreement with Glintborg *et al.* [22] and Thathapudi *et al.* [23]. An abnormal sex steroid milieu also has a potential cause to the chief pathophysiology of MS in PCOS. Androgen excess may serve as an endocrine modulator of MS thus playing another key role beyond its role in ovulatory dysfunction.

In the present study, higher mean testosterone value was recorded in PCOS cases compared with controls (mean=0.44±0.60 and o.24±0.13 ng/ml), respectively. However, this difference did not reach statistical significance.

These results are in agreement with Ravi *et al.* [24], who reported higher mean testosterone value in PCOS cases compared with controls with no statistically significant difference.

Pikee *et al.* [21] reported higher levels of testosterone in PCOS patients compared with controls. Moreover, another study conducted by Moran *et al.* [25] showed that basal testosterone in obese PCOS cases was significantly more than nonobese PCOS patients.

Insignificant differences observed in the present study may be due to the matching of cases and controls as regards BMI, in addition to the heterogeneity of our sample which included mild, moderate, and severe PCOS patients.

It was suggested that irisin is released from the skeletal muscle in response to exercise. Irisin also promotes browning of white fat in humans so that it might be considered a health-promoting hormone [26].

Many functions for irisin were proposed, involving arterial plaque buildup reduction through preventing the inflammatory cells accumulation, leading to decreasing the risk of atherosclerosis [27], raising energy expenditure in the myocardium [28], promoting mitochondrial biogenesis [29], insulin sensitivity, and utilization of the glucose into muscle tissues [30].

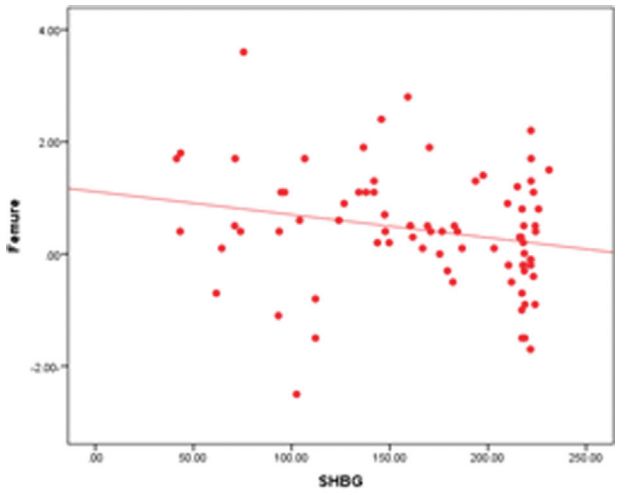
Receiver operating characteristic curve was done and demonstrated that the cut-off value for irisin level in our study was greater than 0.161 μg/dl.

Table 2 Correlation between bone mineral density and the studied parameters in the polycystic ovary syndrome group

All patients	Spine		Femur	
	r	*P* value	*r*	*P* value
Age (year)	0.110	0.334	0.125	0.269
Height (cm)	0.213	0.057	0.003	0.980
Weight (kg)	0.286*	0.010	0.399**	0.000
BMI (kg/m^2)	0.258*	0.021	0.420**	0.000
WC (cm)	0.347**	0.002	0.423**	0.000
SBP (mmHg)	−0.064	0.571	−0.032	0.776
DBP (mmHg)	−0.044	0.696	−0.073	0.522
mFG score	−0.018	0.893	0.024	0.857
FPG (mg/dl)	−0.049	0.663	0.051	0.656
Ca (mg/dl)	−0.040	0.727	−0.110	0.333
Phosphorus (mg/dl)	−0.046	0.688	−0.068	0.550
Cholesterol (mg/dl)	0.059	0.602	0.085	0.454
TG (mg/dl)	0.019	0.865	0.018	0.876
HDL (mg/dl)	−0.071	0.530	−0.110	0.333
LDL mg/dl	−0.038	0.736	0.172	0.126
TG/HDL ratio	0.050	0.660	0.087	0.443
FSH (mU/ml)	0.033	0.773	0.026	0.820
LH (mU/ml)	−0.049	0.665	0.038	0.739
Prolactin (ng/ml)	−0.126	0.265	0.107	0.346
E2 (pg/ml)	0.105	0.353	0.016	0.885
TSH (μIU/ml)	0.063	0.578	−0.221*	0.049
FT4 (ng/dl)	0.121	0.283	0.187	0.097
Insulin (IU/ml)	0.251*	0.025	0.307**	0.006
HOMA-IR	0.201	0.074	0.267*	0.017
Irisin (μg/dl)	0.249*	0.026	0.305**	0.006
TT (nmol/l)	0.032	0.780	0.173	0.125
SHBG (nmol/l)	−0.131	0.245	−0.225*	0.045
FAI	0.074	0.515	0.240*	0.032

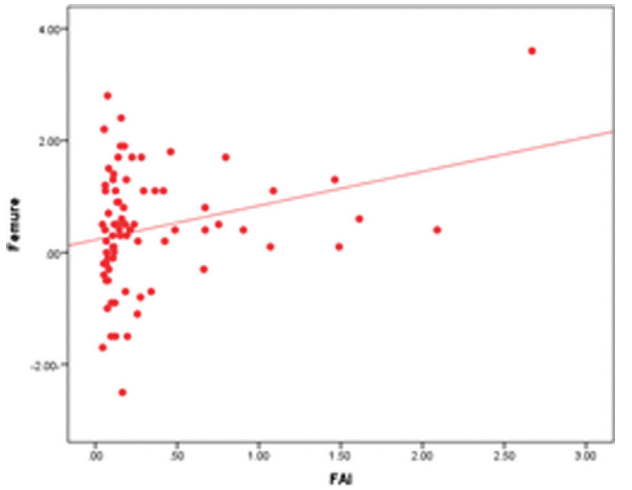
Ca, calcium; DBP, diastolic blood pressure; E2, estradiol; FAI, free androgen index; FPG, fasting plasma glucose; FSH, follicular stimulating hormone; FT4, free thyroxin; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance; LDL-C, low-density lipoprotein cholesterol; LH, luteinizing hormone; mFG, modified Ferriman–Gallwey score; SBP, systolic blood pressure; SHBG, sex hormone binding globulin; TG, triglyceride; TSH, thyroid stimulating hormone; TT, total testosterone; WC, waist circumference.

Figure 3



Correlation between *z*-score of the femur and sex hormone binding globulin.

Figure 4



Correlation between *z*-score of the femur and free androgen index.

In the present study, fasting irisin level was significantly higher in the PCOS group compared with the control group (*P*=0.000). These results are in agreement with Chang *et al.* [31], Pukajlo *et al.* [32], and Bostanci *et al.* [33]. Lean mass increase which may be induced by elevated levels of androgens in PCOS women might contribute to irisin secretion [34].

The case–control study conducted in Egypt by Foda *et al.* [5] was carried out on 80 cases with PCOS and 80 controls. They found that the serum irisin levels were significantly higher in the PCOS group compared with the control group.

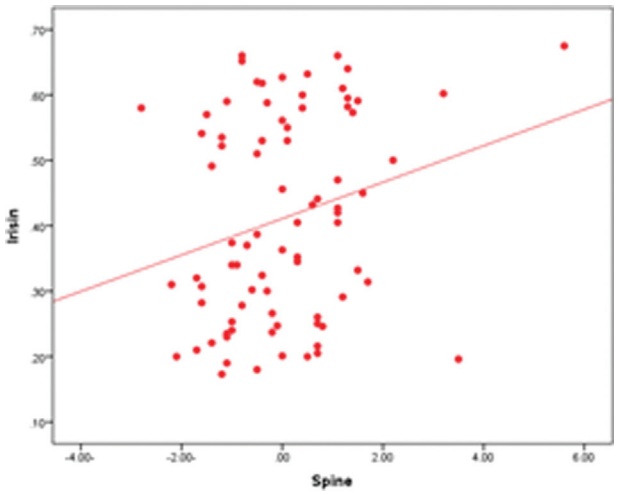
The high circulating levels of irisin in PCOS patients can be explained as an irisin resistance state similar to IR [31,35]. Irisin resistance may occur in PCOS women similar to leptin resistance observed in obesity and metabolic syndrome patients [33].

As irisin normally raises energy expenditure and induces browning of white adipose tissue [4], it is also suggested that elevated irisin level acts as a protective mechanism to overcome obesity commonly associated with PCOS.

PCOS may have an impact on bone metabolism. Strong data on BMD and fracture risk in PCOS are lacking. The varied components of the syndrome may influence bone mass through interrelated metabolic pathways.

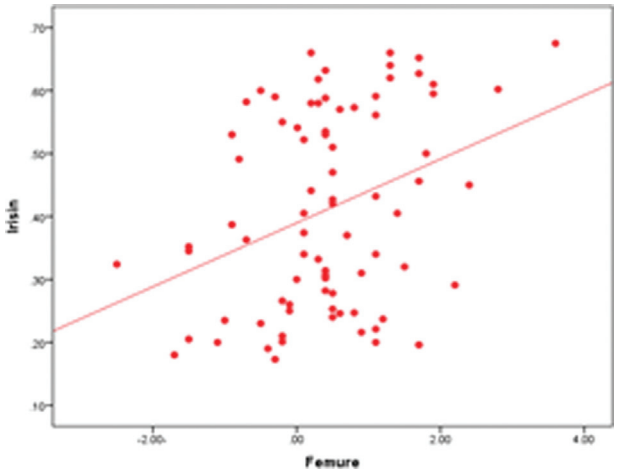
In the present study, BMD of the femur and spine was found to be higher in PCOS group compared to control group (*P*=0.000, 0.044), respectively.

Figure 5



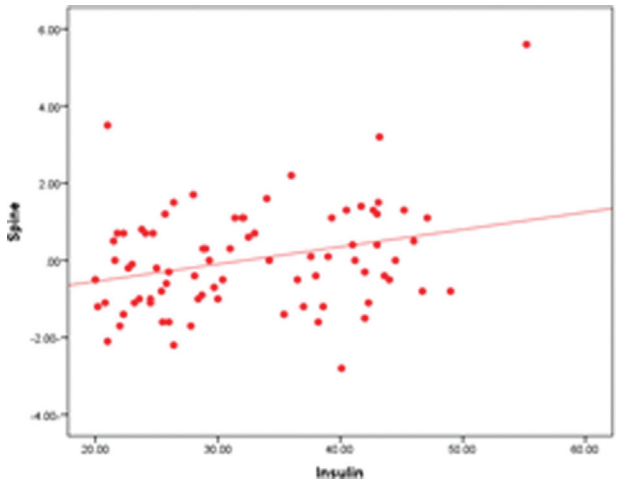
Correlation between *z*-score of the spine and serum irisin level.

Figure 6



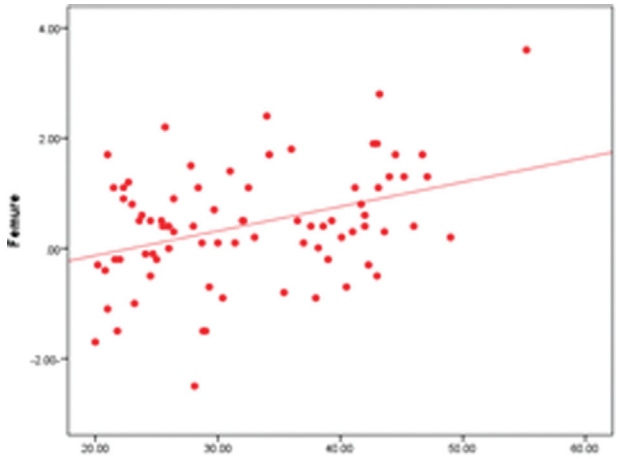
Correlation between *z*-score of the femur and serum irisin level.

Figure 7



Correlation between *z*-score of the spine and insulin.

Figure 8



Correlation between *z*-score of the femur and insulin.

These findings are in agreement with Di Carlo *et al.* [36], Zborowski *et al.* [37], and Glintborg *et al.* [38].

In a study performed by Glintborg *et al.* [38] on Caucasian, reproductive-aged, hirsute patients, they demonstrated significantly higher BMD levels compared with controls. However, another study performed by Noyan *et al.* [39] showed that BMD measurements were not different between the patients with PCOS and healthy control women.

In the current study, there was a positive correlation between BMD and weight, BMI, insulin and HOMA-IR in the PCOS group.

These results are in agreement with Katulski *et al.* [8] who concluded that BMI is one of the most important determinants of BMD in PCOS.

Bonewald and Johnson [40] suggested that mechanical loading increases bone formation by declining apoptosis and growing proliferation and differentiation of osteocytes and osteoblasts.

Possible mechanisms of the positive action of excessive body weight on BMD include the increase of the biomechanical forces exerted on the bone, the increased conversion of androgens to estrogen by the adipocytes, and the IR usually associated with obesity. Hyperinsulinemia stimulates osteoblast activity directly and indirectly via the suppression of SHBG and insulin-like growth factor-binding protein (IGFBP) production [41].

Suppressed SHBG and IGFBP concentrations may be responsible for the elevated bioavailable sex hormones and insulin-like growth factor (IGF). Insulin also

stimulates the production of hepatic IGF-1 [41]. Therefore, insulin resistance and adiposity might act synergistically on bone metabolism and increase bone formation.

PCOS is also associated with hyperandrogenism. Androgens can influence bone directly by the activation of androgen receptors (ARs), or indirectly after peripheral conversion into estrogens within the extraglandular tissues with subsequent activation of estrogen receptors (ERs).

Previous studies had reported inconsistent results regarding the impact of hyperandrogenism on bone mass in PCOS [8,42].

Osteoblasts and osteoclasts all express both ARs and ERs, with a predominance of ARs on osteoblasts [37]. It has been also proposed that androgens may decrease IL-6 production, constrain the production of prostaglandins, and inhibit parathyroid hormone action on osteoblasts [37].

Hyperandrogenism increases muscle mass and hence it has been assumed to affect BMD indirectly via increasing the skeletal loading [34].

In the present study, however, there was no correlation between BMD and testosterone level. And these results are in agreement with Adami *et al.* [43], Good *et al.* [44], and Berberoglu *et al.* [45]. There were statistically significant correlations between BMD of femur and markers of androgen status (FAI and SHBG), (r=0.24, P=0.032), and (r=−0.225, P=0.045), respectively.

Bone mass might be affected by interrelated metabolic events that are not necessarily mediated by androgens. Understanding this cross-regulation between bone and energy metabolism may offer a novel endocrine standpoint on bone metabolism in PCOS. Additional long-term prospective studies of BMD in PCOS patients in the postmenopausal period are required.

It was also found that irisin could enhance osteoblast differentiation and increase bone cortical thickening and trabecular volume in mice *in vivo* [46]. So irisin may be a muscle-derived bone anabolic hormone and a useful biomarker for bone metabolism.

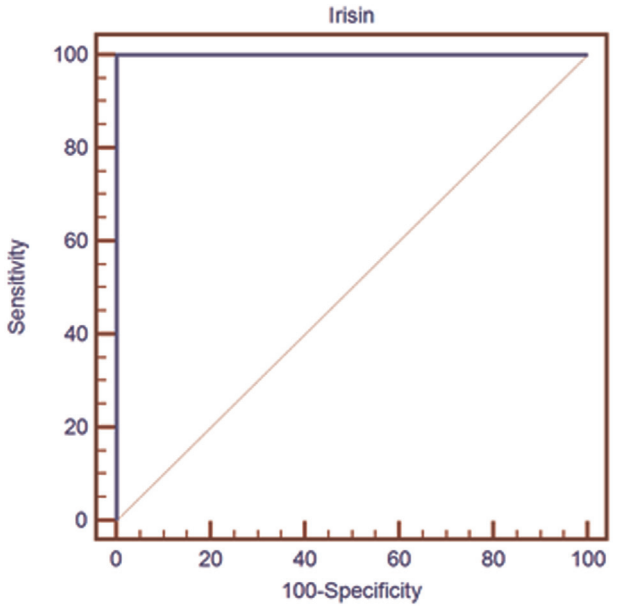
In the present study, fasting irisin level was positively correlated with BMD of the spine (r=0.249, P=0.026)

Table 3 Receiver operating characteristic curve for serum irisin level of the study population

Cut-off point	AUC	Sensitivity	Specificity	PPV	NPV
>0.161	1.000	100.00	100.00	100.0	100.0

AUC, area under the curve; NPV, negative predictive value; PPV, positive predictive value

Figure 9



Receiver operating characteristic curve for serum irisin level of the study population.

Table 4 Linear regression analysis estimating the effects of studied variables on z-score of the spine in the polycystic ovary syndrome group

	Unstandardized coefficients		Standardized coefficients	t	P value
	B	SE	β		
(Constant)	−6.91	1.499		−4.608	0.000
Weight (kg)	−0.003	0.012	−0.044	−0.282	0.779
BMI (kg/m^2)	−0.009	0.06	−0.037	−0.157	0.875
WC (cm)	0.061	0.028	0.44	2.159	**0.034**
Insulin (IU/ml)	0.243	0.116	1.572	2.103	**0.039**
Irisin (mg/dl)	−13.455	6.078	−1.556	−2.214	**0.030**

WC, waist circumference. Bold values for the significant predictors for z-score of the spine.

and femur (r=0.305, P=0.006), respectively. These results are in agreement with Colaianni *et al.* [7], Katulski *et al.* [8], and Singhal *et al.* [47].

In a study performed by Singhal *et al.* [47] who studied 85 adolescent women (38 amenorrheic athletes, 24 eumenorrheic athletes, and 23 nonathletes) showed positive correlation between irisin and BMD.

However, another study conducted by Gao *et al.* [1] showed a negative correlation between serum irisin levels and BMD in the PCOS group. They also found no correlations between irisin, BMI, and age.

Linear regression analysis revealed that serum irisin level is the most significant predictor for z-score of the spine in the PCOS group followed by WC and fasting serum insulin, in that sequence.

This might provide an innovative milestone to the biological suggestion supporting the tight relationship between skeletal muscle myokines and the skeleton. Further research examining the role of irisin in mediating muscle bone connectivity is needed.

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Conflicts of interest
There are no conflicts of interest.

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