Original Article

Serum Irisin in Polycystic Ovary Syndrome and its Alteration with Metformin Intervention

Radhakrishna Telagareddy, Padala Ravi Kumar, Sudhi Ranjan Pattanaik, Deepak Kumar Dash, Debasish Patro, Bijay K. Sahoo, Mahija Sahu[1]

Departments of Endocrinology and [1]Gynaecology and Obstetrics, M.K.C.G Medical College, Berhampur, Odisha, India

Abstract

Introduction: Studies investigating the alterations of serum irisin and its change with metformin therapy in patients with polycystic ovary syndrome (PCOS) are conflicting. Our aim is to study serum irisin in PCOS patients and the change of irisin levels with metformin therapy over 6 months. **Methods:** This is a randomized control study conducted in 187 PCOS cases and 94 age-matched controls aged 18–40 years. Detailed evaluation of anthropometric, biochemical, and hormonal parameters was performed. A subset of 99 overweight/obese patients with body mass index (BMI) ≥23 kg/m^2 were stratified into a metformin group (n = 67) receiving 500 mg thrice daily and a lifestyle intervention-only group (n = 32). The effect of metformin therapy on serum irisin levels was measured at the end of 6 months. Statistical analyses were performed with SPSS version 26.0 Software. **Results:** Serum irisin was higher in PCOS patients than in controls [12.47 (8.1–17.7) vs 8.3 (7.0–9.6) ng/ml, $P < 0.001$], independent of BMI. Serum irisin showed a significant positive association with BMI (β =0.168), waist-to-hip ratio (β =0.166), leutinizing hormone (β =0.225), TG (β =0.305), FAI (β =0.151), and testosterone (β =0.135). Serum irisin showed a significant positive association with homeostatic model assessment of insulin resistance (HOMA-IR) (β =0.14, $P = 0.04$) in overweight/obese PCOS patients only (n = 146) but not in the whole PCOS cohort (n = 187). Metformin reduced the median serum irisin levels significantly (13.9 to 12.1 ng/ml, $P < 0.001$), and the delta change in irisin levels was associated with HOMA-IR in the metformin group. **Conclusion:** Serum irisin is increased in PCOS patients independent of BMI. Metformin therapy reduced serum irisin levels in overweight/obese PCOS patients by improving insulin resistance.

Keywords: Insulin resistance, irisin, metformin, polycystic ovary syndrome

Introduction

Polycystic ovary syndrome (PCOS) is a common endocrinopathy affecting 5–18% of the women in the reproductive age group.[1] Insulin resistance (IR) and hyperandrogenemia are the cardinal features of PCOS. Although insulin resistance is not a diagnostic criterion for PCOS, it is found in 44–70% of women with PCOS.[2] The diagnosis of PCOS is based on revised Rotterdam 2003 criteria.[3] However, in adolescence, polycystic ovarian morphology (PCOM) criteria are not recommended for diagnosis of PCOS.[4] So diagnostic markers which are independent of these like anti-Mullerian hormone (AMH) and irisin have been proposed for the diagnosis of PCOS.[5-7]

Irisin is a 12 Kd adipomyokine and is secreted into the circulation after proteolytic cleavage of fibronectin type III domain-containing protein 5 (FNDC5). It was discovered by Bostrom *et al*.[8] in 2012. In mice studies, it was shown to increase energy expenditure, weight loss, and browning of adipose tissue.[8] Its exact physiological role in humans still needs to be elucidated.[8] Studies on irisin in PCOS patients have been contradictory with some showing increased[9-15] irisin, while others showing decreased[6,16] or similar[17,18] levels compared to controls. Some studies have shown that irisin levels in PCOS patients correlated[10,12] with body mass index (BMI), while others were contradictory.[6,17,18] Similarly, the correlation of irisin with insulin resistance in PCOS patients was different in studies. Meta-analysis by Cai *et al*.[19] in 2018 of eight studies could not find a

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Address for correspondence: Dr. Padala Ravi Kumar, Department of Endocrinology, M.K.C.G Medical, College, Berhampur, Odisha - 760 004, India.
E-mail: padala797@gmail.com

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correlation between irisin and homeostatic model assessment of insulin resistance (HOMA-IR), whereas a meta-analysis by Bacopoulou *et al*.[20] in 2020 of 11 studies found a significant correlation between serum irisin and HOMA-IR. So, there is a need to study serum irisin in PCOS patients, especially with regard to obesity, BMI, and insulin resistance.

Insulin resistance is central to the pathogenesis of PCOS.[2] Studies reducing the insulin resistance with metformin on irisin levels have also shown either decreased[11,21] or no effect.[22] So, the present study proposes to study the effect of metformin in a subset of overweight/obese PCOS patients over 6 months for any effect on serum irisin.

Materials and Methods

This study was undertaken among 187 PCOS patients aged 18–40 years, attending the outdoor clinics of the Department of Endocrinology and Obstetrics and Gynecology of a tertiary care center from July 2021 to January 2023.

Newly detected PCOS patients who fulfilled revised Rotterdam 2003 criteria were included based on the presence of any two out of the three features like (i) oligo or anovulation (ii) hyperandrogenism or hyperandrogenemia, and (iii) polycystic ovarian morphology, which includes ≥12 follicles of size 2 to 9 mm or volume ≥10 ml in either of the ovaries. The cut-off for defining hyperandrogenism was taken as modified Ferriman-Gallaway score (mFG score) ≥8; hyperandrogenemia was defined as serum total testosterone ≥60 ng/dl. Other causes for hyperandrogenemia and ovulatory dysfunction including non-classical congenital adrenal hyperplasia (NCCAH), Cushing's syndrome, hypothyroidism, and hyperprolactinemia were excluded. Patients with diabetes mellitus have been excluded as it was shown to affect serum irisin in prior studies.[23] Patients on any medications affecting glucose/lipid/androgen metabolism and insulin sensitivity like glucocorticoids, oral contraceptive pills (OCPs), and antiandrogens within past 3 months were excluded from the study.

Ninety-four age-matched healthy females who had regular menstrual cycles without features of hyperandrogenism served as controls in the present study. In all the cases and controls, a detailed history was taken and anthropometric measures including height, weight, BMI (kg/m^2), waist circumference (WC), hip circumference (HC), and waist-to-hip ratio (WHR) were carried out using standard procedures. Blood pressure was measured using a mercury sphygmomanometer in the right upper arm in the sitting position. The presence of acanthosis nigricans was noted, and hirsutism was assessed by modified Ferriman-Gallaway score. Clinical examination was done in the presence of a female staff nurse ensuring the privacy of the individuals.

The cases and controls were segregated according to BMI into an overweight/obese group (BMI ≥23 kg/m^2) and a normal weight group (BMI <23 kg/m^2) according to Asian criteria.[24] In all the subjects after overnight fasting of at least 8 hours, blood was collected for estimation of fasting plasma glucose (FPG), HbA1C, serum insulin, lipid profile, serum creatinine, serum urea, liver function tests, and hormonal parameters like thyroid stimulating hormone (TSH), prolactin (PRL), leutinizing hormone (LH), FSH, testosterone and dehydroepiandrosterone sulfate (DHEAS), AMH, sex hormone binding globulin (SHBG), and androstenedione. A separate 4 ml venous blood was collected, centrifuged at 2000 rpm, and stored at a temperature of -80^0 C for estimation of serum irisin. Oral glucose tolerance test (OGTT) was performed in all the cases and controls with 75 grams of anhydrous glucose, and plasma glucose was measured at 2 h for 2 hr-post glucose plasma glucose (2 hr PGPG).

FPG, 2 hr PGPG, fasting lipids, serum creatinine, urea, bilirubin, ALT, and AST were measured using a biochemical autoanalyzer (Siemens Autopak 300 APK). HbA1C was estimated by the high-performance liquid chromatography (HPLC) method based on ion exchange. Hormones TSH, prolactin, FSH, LH, testosterone, DHEAS, insulin, AMH, SHBG, and androstenedione were measured by a CLIA method using a Seimens Advia Centaur CP machine.

Assay for irisin: Serum irisin was measured by using ELISA kits (CUSABIO from Krishgenbiogenics, catalog number: CSB-EQ027943HU). The intra- and inter-assay coefficients of variation were <8% and <10%, respectively, with an assay sensitivity of 1.8 ng/ml, and were analyzed by using an ELISA reader (Varioskan Lux thermo scientific reader) at 450 nm.

Intervention study

Out of 187 PCOS cases, 146 were overweight or obese (BMI ≥23 Kg/m^2) patients. Out of these 146 patients, 99 were recruited for the intervention study after exclusion of patients planning for pregnancy, in need for OCP, anti-androgens [67 PCOS patients to the metformin therapy group (MF group) and 32 to the lifestyle modification only group (LSM group)] by stratified randomization. Allocation concealment was done, and the allocator was blinded to the intervention. Fifty-two cases in the MF group and 25 in the LSM group completed the study at the end of 6 months (15 people in the MF group and 7 in the LSM group were excluded for reasons like pregnancy/consent withdrawal/GI intolerance/need for OCP) as illustrated in Figure 1. In the MF group, metformin was started at a dose of 500 mg twice daily and increased to 500 mg thrice daily after 2 weeks. In both MF and LSM groups, lifestyle measures were advised, which included moderate aerobic exercise of at least 150 min/week and caloric restriction aimed to lose weight of at least 3–5% of body weight. At the end of 6 months, the clinical and anthropometric examinations were done in both MF and LSM groups and blood was collected for detailed analysis as previously described for biochemical and hormonal parameters including serum irisin to study any change in them by using per-protocol analysis. The analyzer was blinded to the intervention.

Statistical analysis

The sample size was calculated to detect a difference of 2.8 ng/ml in irisin levels between PCOS cases and controls [a

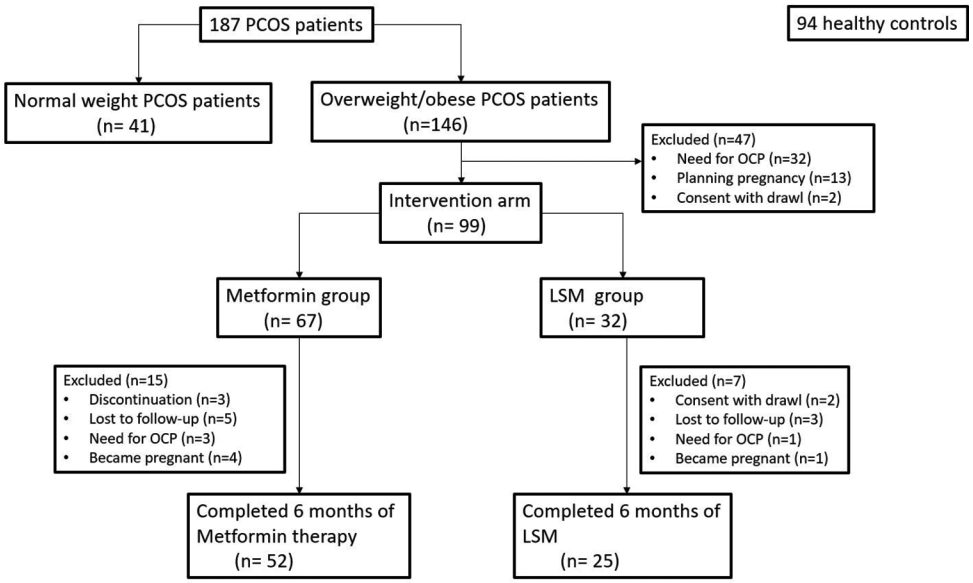

Figure 1: Outline of the study. GI intolerance, gastro-intestinal intolerance; OCP, oral contraceptive pills

standard deviation (SD) of 4.8 and 3 ng/ml, respectively].[15] The sample size was estimated to be 54 in each group. Calculations were done based on 95% confidence levels and 95% power using Open Epi software, Version 3.01.

Normality distribution for continuous variables was tested by using Kolmogorov–Smirnov test. Data (parametric) were expressed as mean ± SD, whereas data (non-parametric) were expressed as median (P25–P75). Non-parametric tests (Mann–Whitney U test) and parametric tests (independent *t*-test) were used for inter-group comparison. Intra-group comparison was done by using Wilcoxson rank test and paired *t*-test for non-parametric data and parametric data, respectively. Spearman's and Pearson's correlation coefficients were used to analyze the correlation between different parameters for non-parametric and parametric data, respectively. Multiple regression analysis was performed to see the independent association of variables. The receiver operating characteristic curve (ROC) was generated to find the diagnostic utility of various parameters. *P* value < 0.05 was considered statistically significant. Statistical analyses were performed with SPSS version 26.0 Software.

Ethical aspects

The study was aproved by Institutional ethics committee vide letter number-869, dated 20.07.2021 of M.K.C.G Medical College,Berhampur. All the participants gave written informed consent.

Results

In the present study, there were 187 cases of PCOS and 94 age-matched healthy controls as shown in Figure 1. Overweight/obese and normal weight subjects constitute 78% (n = 146) and 22% (n = 41) in cases and 46% (n = 43) and 54% (n = 51) in controls, respectively. The comparison of baseline characteristics of PCOS cases and controls is shown in Table 1. In comparison to the controls, PCOS patients had a significantly higher weight, BMI, WHR,

Table 1: Comparison of baseline characteristics between total PCOS cases and controls

Variable	Cases (*n*=187)	Controls (*n*=94)	*P*
Age (years)	23.3 (20.6-27)	23 (20.7-26.2)	0.31
BMI (kg/m^2)	25.2 (23.5-27.5)	23.3 (20.6-25.5)	<0.001
WHR	0.85 (0.8-0.88)	0.79 (0.73-0.86)	<0.001
SBP (mmHg)	120 (110-126)	114 (110-118)	<0.001
DBP (mmHg)	76 (70.1-80)	76 (70-78)	0.25
FPG (mg/dl)	88.9 (84-94)	79 (75-82)	<0.001
2HPGPG (mg/dl)	124 (116-131)	101 (95.3-116)	<0.001
Fasting insulin (mIU/L)	11.2 (6.1-16)	1.8 (1.3-3.2)	<0.001
HOMA-IR†	2.4 (1.3-3.7)	0.35 (0.26-0.60)	<0.001
HbA1C (%)	5.5 (5.3-5.6)	5.2 (5.1-5.4)	<0.001
TC (mg/dl)	184 (156-213)	126 (117-134)	<0.001
TG (mg/dl)	145 (112-215)	112 (93-132)	<0.001
HDL (mg/dl)	45 (40-53)	46 (43-54)	0.04
LDL (mg/dl)	100.6 (79.8-127.2	55.8 (50.4-66.6)	<0.001
TSH (mIU/L)	2.1 (1.5-2.6)	1.8 (1.5-2.3	0.14
FSH (IU/L)	4.7 (3.3-6.8)	4.13 (3.3-5.6)	0.06
LH (IU/L)	10.9 (7.3-14.9)	5.9 (1.9-8.1)	<0.001
PRL (ng/ml)	10.5 (7.5-14.4)	9.4 (7.9-12.5)	0.27
Testosterone (ng/dl)	37.7 (23.2-59.0)	13.9 (12.2-17.8)	<0.001
Androstenedione (ng/ml)	4.3 (2.7-5.9)	2.5 (1.9-3.4)	<0.001
SHBG (nmol/L)*	49.4±24.2	52.7±15	0.16
DHEAS (ng/ml)	210 (138.1-271.9)	167 (133.4-203.2)	0.002
FAI‡	2.9 (1.9-5.0)	0.98 (0.75-1.22)	<0.001
AMH (ng/ml)	8 (6.4-10)	3.6 (3.3-4.2)	<0.001
Irisin (ng/ml)	12.47 (8.1-17.7)	8.3 (7.0-9.6)	<0.001

*Expressed as mean±SD, rest of the values are expressed as median [Inter quartile range (IQR)]. †HOMA-IR was calculated by using the formula: Fasting Insulin (mIU/L) × FPG (mg/dl)/405. ‡Free androgen Index (FAI) was calculated by total testosterone/SHBG X 100 (both in nmol/L)

SBP, FPG, 2 hr PGPG, HbA1c, fasting insulin, HOMA IR, lipids (TC, LDL, TG), PRL, LH, FSH, testosterone, FAI, AMH, and DHEAS. HDL was significantly lower in PCOS cases compared to controls. However, SHBG was not different between cases and controls. Testosterone and androstenedione levels were significantly higher in PCOS cases compared to controls [37.7 (23.2–59) vs 13.9 (12.2–17.8) ng/dl, median (IQR) $P < 0.001$ and (4.4 ± 2.1 vs 2.7 ± 1.2, ng/ml, $P < 0.001$)], respectively. FAI was significantly higher in PCOS patients than in controls [2.9 (1.9–5.0) vs 0.98 (0.75–1.22), $P < 0.001$]. HOMA-IR was around six times higher in PCOS cases than in controls.

Serum irisin

Serum irisin was significantly higher in PCOS patients, compared to controls [12.47 (8.1–17.7) vs 8.3 (7.0–9.6) ng/ml, $P < 0.001$] [Table 1; Figure 2a]. The serum irisin remained elevated even when BMI was matched between cases and controls [13 (8.5–18.1) vs 8.2 (6.8–10) ng/ml, $P < 0.001$] as shown in Figure 2b. Sub-group analysis revealed that serum irisin was significantly higher in overweight/obese PCOS patients (BMI ≥ 23 kg/m^2) compared to normal weight PCOS patients (BMI < 23 kg/m^2) [13.45 (8.9–19) vs 9.8 (6.9–12.6) ng/ml, $P < 0.001$] as shown in Figure 2c. Comparison of overweight/obese PCOS cases with overweight/obese controls and normal weight PCOS cases with normal weight controls revealed serum irisin still remained significantly high in cases compared to controls as shown in Figure 2 d-e.

Correlations of irisin with other variables

The bivariate correlation analysis in PCOS patients revealed that serum irisin had a significant positive correlation with weight (r = 0.28, $P < 0.001$), BMI (r = 0.33, $P < 0.001$), WHR (r = 0.2, $P = 0.005$), SBP (r = 0.17, $P = 0.01$), FPG (r = 0.31, $P < 0.001$), 2 hrPGPG (r = 0.17, $P = 0.01$), insulin (r = 0.19, $P = 0.007$), HOMA-IR (r = 0.25, $P < 0.001$), TC (r = 0.24, $P < 0.001$), TG (r = 0.46, $P < 0.001$), LH (r = 0.41, $P < 0.001$),

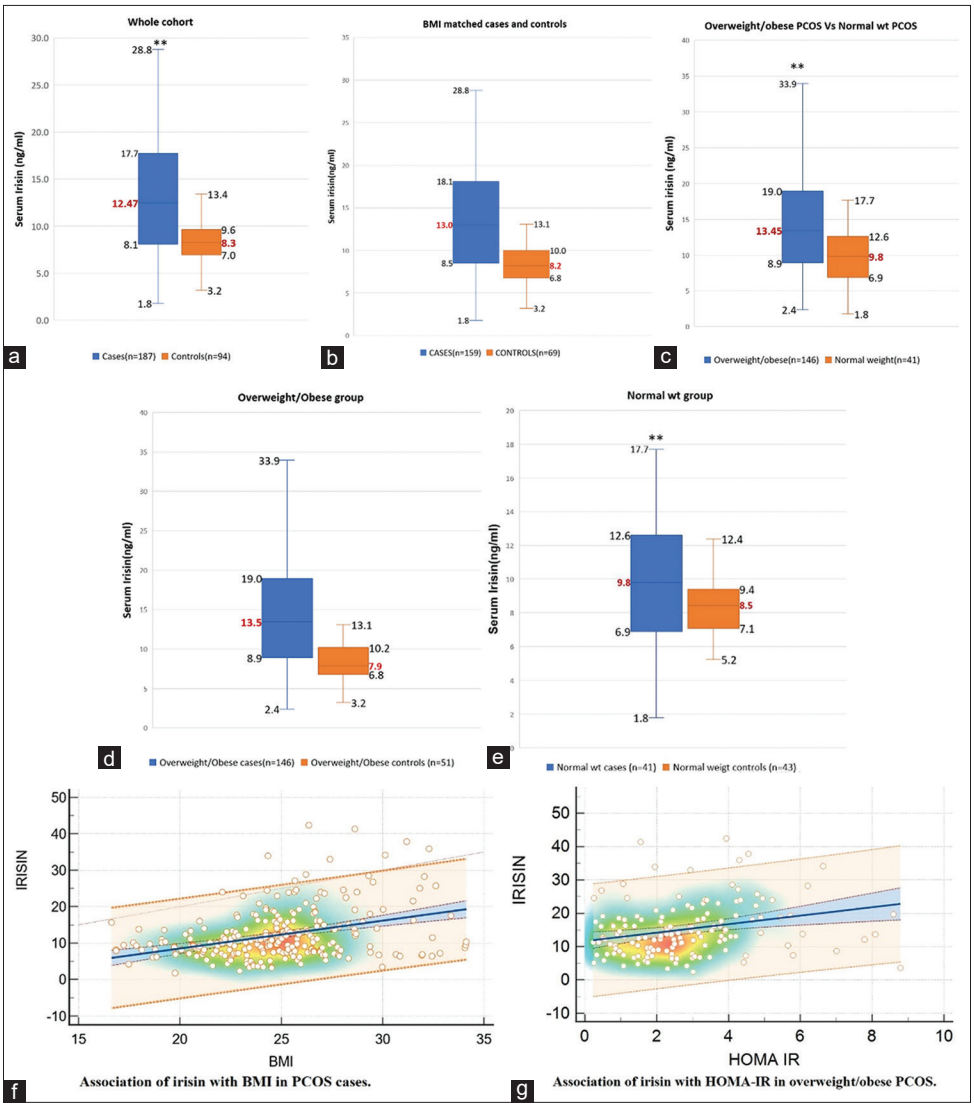

Figure 2: Comparison of serum irisin levels across various groups (a-e); association of irisin with BMI in whole PCOS cases (n = 187) (f); association of irisin with HOMA-IR in overweight/obese PCOS (n = 146) (g). ** Statistically significant difference ($P < 0.05$)

testosterone (r = 0.27, $P < 0.001$), FAI (r = 0.38, $P < 0.001$), and AMH (r = 0.23, $P < 0.001$) and a significant negative correlation with HDL (r = -0.19, $P = 0.007$). However, multiple regression analysis for irisin showed only a significant positive association with BMI (β =0.168, $P = 0.007$) as shown in Figure 2f, WHR (β =0.166, $P = 0.006$), LH (β =0.225, $P < 0.001$), TG (β =0.305, $P < 0.001$), FAI (β =0.151, $P = 0.02$), and testosterone (β =0.135, $P = 0.03$) [Table 2]. To see whether HOMA-IR was significantly associated with irisin, regression analysis was also done in obese PCOS patients (n = 146), which showed a significant positive association of irisin with HOMA IR (β =0.14, $P = 0.04$) [Figure 2g], which was not observed in the whole cohort (n = 187).

Effect of metformin on serum irisin in PCOS patients

In the intervention group, 52 patients in the MF group and 25 patients in the LSM group completed 6 months of the study protocol. Analysis was done by the intention-to-treat method. At baseline, the MF group and LSM group had comparable anthropometric, biochemical, and hormonal characteristics.

Comparison between various parameters before and after 6 months of metformin therapy showed a significant reduction in weight, BMI, FPG, 2 hrPGPG, TC, TG, fasting insulin, HOMA-IR, AMH, LH, testosterone, FAI, and DHEAS as well as a significant increase in HDL and SHBG levels in the MF group [Table 3]. However, there was no significant reduction in any of the variables in the LSM-only group, except for serum prolactin, AMH. At the end of 6 months, there was a significant reduction in serum irisin levels in the MF group [13.9 (10.46–20.42) to 12.1 (7.9–15.5) ng/ml, $P < 0.001$] [Table 3, Figure 3a], while the reduction was non-significant in the LSM-only group [14.8 (11.5–18.7) to 14.1 (11.4–18.1) ng/ml, $P = 0.09$]. Moreover, the delta change in serum irisin was also significantly different between the MF and LSM groups [-2.3 (-4.2,-1.0) vs -0.4 (-1.05,-0.1) ng/ml, $P < 0.001$]. Delta change in irisin is well correlated with change in HOMA-IR [Figure 3b], TG, and LH. In multiple regression analysis, the delta change in irisin levels was significantly associated only with the delta change in HOMA-IR (β =0.658, $P < 0.001$) in the metformin intervention group.

Table 2: Multiple regression analysis of variables associated with serum irisin in PCOS cases (*n*=187)

Parameter	Standardized beta coefficient	
	β	*P*
BMI	0.168	0.007
WHR	0.166	0.006
TG	0.305	< 0.001
LH	0.225	< 0.001
Testosterone	0.135	0.029
FAI	0.151	0.02

Discussion

In the present study, serum irisin was found to be increased in PCOS cases compared to age-matched controls independent of BMI, but it was more marked in overweight/obese PCOS cases. It was positively associated with BMI, WHR, LH, TG, testosterone, and FAI but not with HOMA IR in the whole PCOS cohort. However, when sub-group analysis was done in overweight/obese PCOS cases, there was a significant positive association of irisin with HOMA-IR. Metformin therapy for 6 months significantly reduced serum irisin and HOMA-IR.

Serum irisin is a 12 kd adipomyokine and is secreted mostly from the myocytes in response to exercise after cleavage of FNDC5 into the circulation. Serum irisin in PCOS patients has been studied previously, and results varied widely across studies. Serum irisin was shown to be increased in some studies[9-15] similar to the present study, whereas others showed reduced irisin levels[6,16] or similar levels between cases and controls.[17,18] Meta-analysis by Cai *et al*.[19] of eight studies found similarly increased serum irisin levels compared to controls. However, this significance between cases and controls was lost, when BMI was matched contrary to the present study. Another meta-analysis by Bacopoulou *et al*.[20] of 11 studies did not observe raised irisin levels in PCOS women. The variation in results of these studies could be due to heterogeneity in studies, different diagnostic criteria of PCOS, confounding factors like age, BMI, coexisting diabetes mellitus, and medications affecting the insulin sensitivity and androgen levels. The physiological effect of raised serum irisin in PCOS cases may be a metabolic adaptive mechanism to increase the body energy expenditure. In the present study,

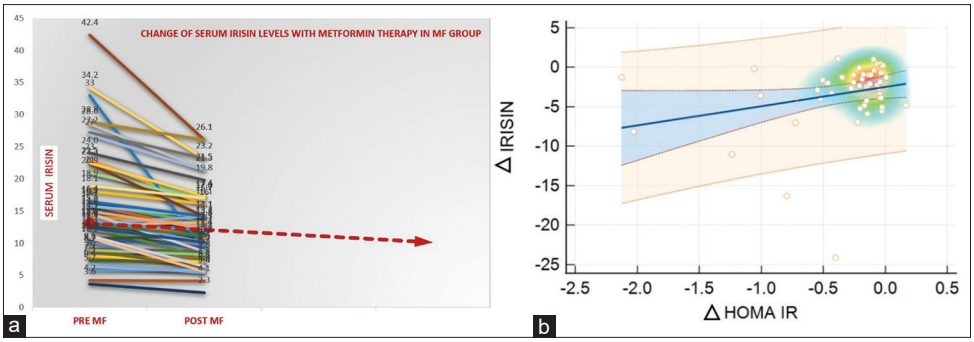

Figure 3: Change of irisin with metformin intervention in MF group (a); association of Δ irisin with Δ HOMA-IR in MF group (b). PRE MF, before starting metformin; POST MF, at 6 months of metformin therapy

Table 3: Comparison of change of various parameters between metformin (MF) group and life style modification only (LSM) group

	MF GROUP (n=52)				LSM GROUP (n=25)				P value for Δ change§
	Initial	End of 6 months	Change	P†	Initial	End of 6 m	Change	P‡	
Weight (Kg)	65.8±8.7	64.6±8.9	-1.2	<0.001	66.2±10.0	66.1±9.0	0.0	0.91	0.011
BMI (Kg/m^2)	26.5±2	26.0±2.1	-0.5	<0.001	26.9±3.2	26.6±2.6	-0.4	0.11	0.68
WHR	0.8±0.05	0.8±0.05	0.0	0.15	0.9±0.1	1.1±1.4	0.3	0.34	0.59
FPG (mg/dl)	90.3±8.2	88.0±6.1	-2.3	<0.001	89.5±7.6	88.0±5.9	-1.5	0.06	0.94
2HPGPG (mg/dl)	124.4±11.5	116.1±23.5	-8.3	0.01	121.9±14.6	118.7±13.6	-3.2	0.22	0.25
Fasting insulin	12.5±8.1	11.2±6.7	-1.3	<0.001	14.9±6.5	14.7±6.3	-0.2	0.27	0.009
HOMAIR	2.8±1.8	2.4±1.47	-0.3	<0.001	3.3±1.4	3.3±1.4	0.0	0.59	0.001
TC (mg/dl)	190.4±43.7	186.5±39.9	-3.9	0.01	186.8±40.6	178.3±29.6	-8.5	0.07	0.87
TG (mg/dl)	186.3±92.6	160.3±67.7	-25.9	<0.001	157.8±79.9	141.9±48.9	-16.0	0.13	0.02
LDL (mg/dl)	108.6±38.2	106.0±34.4	-2.6	0.13	108.6±34.3	101.5±24.4	-7.1	0.06	0.20
HDL (mg/dl)	44.5±8.1	49.5±7.7	5.0	<0.001	46.6±11.6	49.3±8.4	2.7	0.11	0.68
FSH (IU/L)	5.5±3.09	5.3±2.5	-0.3	0.12	5.6±3.2	5.3±2.8	-0.3	0.08	0.75
LH (IU/L)	12.8±5.4	10.6±4.2	-2.2	<0.001	11.5±7.2	10.7±5.7	-0.8	0.2	0.007
PRL (ng/ml)	12.6±7.5	12.1±6.3	-0.5	0.14	11.3±6.9	10.4±6.1	-1.0	0.005	0.06
Testosterone (ng/dl)	39.0±21.8	36.4±19.6	-2.6	0.03	38.5±23.5	36.3±21.0	-2.1	0.10	0.39
Androstenedione (ng/ml)	4.4±2.1	4.3±1.71	-0.1	0.62	4.5±2.0	4.4±1.5	-0.1	0.49	0.56
SHBG (nmol/L)	50.2±25.3	52.6±26.2	2.3	<0.001	51.5±22.0	51.4±21.6	-0.1	0.8	<0.001
FAI*	2.45 (1.6-4.2)	2.15 (1.6-3.3)	-0.3	<0.001	2.65 (1.6-3.9)	2.1 (1.8-3.9)	-0.1	0.09	0.004
DHEAS (ng/ml)	219.8±87.4	206.2±78.5	-13.6	0.04	204.8±85.7	202.8±82.5	-2.0	0.15	0.81
AMH (ng/ml)	8.56±2.76	7.41±2.47	-1.14	<0.001	7.6±2.47	7.16±2.36	-0.44	<0.001	<0.001
Irisin (ng/ml) *	13.9 (10.4-20.4)	12.1 (7.9-15.5)	-2.3	<0.001	14.8 (11.5-18.7)	14.1 (11.4-18.1)	-0.6	0.09	<0.001

*Expressed as median (IQR), rest of the values are expressed as mean±SD. †P-value for change in parameters before and after intervention in MF group. ‡P-value for change in parameters before and after intervention in LSM group. §p-value for delta change in parameters between MF group and LSM group

among PCOS patients, serum irisin was found to be higher in overweight/obese patients compared to normal weight patients. These results were in confirmation with five studies which showed higher irisin levels in the obese group.[9,11,12,18,25] Since irisin is also secreted from adipocytes besides myocytes, it partly explains the increased levels in the overweight/obese group in PCOS. Contrasting these studies, a study by Wang *et al*.[16] found a higher irisin level in normal weight PCOS patients compared to obese PCOS groups. Since BMI could be a confounding factor for raised serum irisin between cases and controls, the present study analyzed irisin after controlling for BMI and it still remained significantly elevated in PCOS cases. Although this is contradicting, these results indicate that factors other than simple obesity may be responsible for the increase in irisin. However, it requires further studies to confirm these findings.

The correlation between serum irisin and HOMA-IR in PCOS patients is different across studies. In the present study, HOMA-IR was significantly correlated with irisin in overweight/obese PCOS patients only but not in the whole cohort. These results are in line with a few studies.[6,15,19] But in contrast, a meta-analysis by Bacopoulou *et al*.[20] in 2020 revealed a moderate correlation between irisin and HOMA-IR (r = 0.372, P = 0.012) in PCOS patients. Similar to hyperinsulinemia in insulin resistance, some authors hypothesized irisin resistance may be responsible for raised irisin levels.[15,26] Whether raised irisin in any way contributes to pathogenesis of PCOS needs to be studied in future studies.

Since insulin resistance plays a major role in pathogenesis of PCOS,[2] serum irisin could serve as a marker of IR.[27] Studies have been conducted with metformin for any change in irisin and HOMA-IR in PCOS cases.[11,21] The present study showed a significant decrease in serum irisin levels in the MF group compared to the LSM group. The delta change in serum irisin correlated with change in HOMA-IR in the metformin group. In an earlier study by Li *et al*.,[11] similar reduction of serum irisin was observed with metformin therapy. However, controls were not included in their study. This reduction in serum irisin with metformin therapy may be attributed to reduction in HOMA-IR and improvement of metabolic milieu. The present study revealed serum irisin is increased in PCOS cases, but its exact role in PCOS needs to be established.

The strengths of our study were a large sample size, segregation and analysis of cases and controls into overweight/obese and normal weight groups, longer duration of intervention arm, and exclusion of various factors that could affect insulin resistance and circulating irisin and comprehensive analysis of various aspects that previous studies on irisin in PCOS may have overlooked. Although the present study is well structured, it has a few limitations like decreased generalizability as it was per-protocol analysis, intervention was blinded only to the analyzer.

Conclusion

Serum irisin is increased in PCOS patients irrespective of BMI. Metformin therapy reduces serum irisin levels in overweight/obese PCOS patients by improving insulin resistance.

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Conflicts of interest

There are no conflicts of interest.

Author contributions

Ravikumar P. conceptualized the study, designed the research, managed recruitment, manuscript editing and proof reading it. Telagareddy R. contributed to recruitment, performed statistical analysis, and participated in manuscript writing. Pattanaik SR. aided in recruitment and intervention. Dash DK. contributed in recruitment, intervention, and proof reading. Allocation concealment was done by Patro D. Sahoo BK and Mahija Sahu contributed to the case recruitment.

Data Availability

The corresponding author of this manuscript is willing to share the data supporting the results of this manuscript upon reasonable request.

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