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Níveis séricos de irisina em mulheres com a Síndrome de Ovários Policísticos: Um estudo de casos e controles

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Esta dissertação de Mestrado segue o formato proposto pelo Programa de Pós-Graduação em Ciências Médicas: Endocrinologia, Faculdade de Medicina, Universidade Federal do Rio Grande do Sul, sendo apresentada na forma de uma revisão geral e um manuscrito sobre o tema da dissertação:

- Revisão: Irisina e Síndrome dos ovários policísticos

- Artigo original: Circulating levels of irisin in Polycystic Ovary Syndrome and control women: a case-control study

SUMÁRIO

RESUMO

Introdução: Irisina é uma adipocina / miocina, descrita pela primeira vez em 2012 e parece estar envolvida na termogênese do tecido adiposo e na homeostase metabólica. A síndrome dos ovários policísticos (PCOS) é reconhecida como um distúrbio endocrinológico prevalente em mulheres com idade reprodutiva, e está frequentemente associado à obesidade abdominal, resistência à insulina, dislipidemia e hipertensão arterial.

Objetivos: Determinar os níveis circulantes de irisina numa amostra de mulheres com PCOS e controles ovulatórias não hirsutas e verificar se os níveis séricos de irisina estão associados com variáveis hormonais, metabólicas e de composição corporal nestas participantes.

Métodos: Neste estudo caso-controle foram incluídas 49 mulheres com PCOS e 33 mulheres controles ovulatórias não-hirsutas com idade e índice de massa corporal (IMC) semelhantes. Variáveis demográficas, antropométricas, hormonais e metabólicas foram obtidas através de dados da história médica, exame físico e dosagens bioquímicas e hormonais convencionais. A composição corporal foi avaliada por absorciometria de raios-X de dupla energia (DXA). Os níveis séricos de irisina foram mensurados por um kit ELISA humano.

Resultados: A pressão arterial sistólica, HOMA, testosterona total e índice de androgênios livres (IAL) foram significativamente maiores e a SHBG foi menor nas PCOS. Após a estratificação por IMC, massa gorda e razão massa gorda / massa magra foram menores em mulheres com peso normal do que em mulheres com sobrepeso / obesidade. O grupo PCOS com peso normal apresentou menos massa magra total do que o grupo PCOS com sobrepeso / obesidade e subgrupos controles. A proporção de massa magra apendicular / IMC foi significativamente maior nas controles de peso normal que em controles com sobrepeso / obesidade, mas os subgrupos de PCOS foram semelhantes entre si e com as controles de peso normal e obesas. Os níveis séricos de irisina foram significativamente maiores nas pacientes

PCOS com sobrepeso / obesidade em comparação com as controles de peso normal. A irisina circulante correlacionou-se positivamente com o HOMA. Observou-se também correlação positiva da irisina com massa magra total e razão massa gorda /massa magra em mulheres com PCOS, mesmo após ajuste para IAL.

Conclusão: Os dados do presente estudo sugerem uma associação de irisina com variáveis de composição corporal.

Palavras-chave: irisina, resistência à insulina, PCOS, DXA.

ABSTRACT

Introduction: Irisin is an adipokine / myokine, first described in 2012 and appears to be involved in adipose tissue thermogenesis and metabolic homeostasis. The polycystic ovary syndrome (PCOS) is recognized as a frequent endocrine disorder in women of reproductive age, and is often associated with abdominal obesity, insulin resistance, dyslipidemia, and hypertension.

Objectives: To determine the circulating levels of irisin in women with PCOS and non-hirsute ovulatory control women, and to evaluate whether serum irisin levels are associated with hormone, metabolic and body composition variables in these participants.

Methods: In this case-control study, 49 women with PCOS and 33 nonhirsute ovulatory controls women with similar age and body mass index (BMI) were enrolled. Demographic, anthropometric, hormone and metabolic variables were assessed by medical history, physical examination and conventional biochemical and hormon determinations. Body composition was assessed by double-energy X-ray absorptiometry (DXA). Serum irisin levels were measured by a human ELISA kit.

Results: Systolic blood pressure, HOMA, total testosterone and FAI were higher and SHBG was lower in PCOS. After stratification by BMI, fat mass and fat mass / lean mass ratio were lower in women of normal weight in overweight / obese women. The PCOS group at normal weight had less total lean mass than the overweight / obese PCOS group and control subgroups. The lean appendicular mass / BMI ratio was significantly higher in normal weight controls than in overweight / obese controls, but PCOS subgroups were similar between them and with normal and obese weight controls. Serum irisin levels were significantly higher in overweight / obese PCOS patients than in normal weight controls. Circulating irisin was positively correlated with HOMA. A positive correlation was also observed between irisin

and total lean mass and fat mass / lean mass ratio in women with PCOS, even when adjusted for FAI.

Conclusion: Our data suggest an association of irisin and body composition variables.

Keywords: irisin, insulin resistance, PCOS, DXA.

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Revisão da literatura: Irisina e Síndrome dos ovários policísticos

A irisina é uma miocina/adipocina (Panati *et al.*, 2016), descoberta em 2012, por Broston et al. e descrita como uma proteína termogênica, reguladora do metabolismo do tecido adiposo e da homeostase da glicose (Boström *et al.*, 2012; Boström *et al.*, 2014). De acordo com a literatura a irisina seria capaz de induzir, através de reações bioquímicas, a comunicação entre o tecido muscular o tecido adiposo (Erickson, 2013; Novelle et al., 2013; Irving, Still e Argyropoulos, 2014) induzindo o tecido adiposo branco a se comportar como tecido adiposo marrom, promovendo a termogênese e consequente perda de peso (Boström *et al.*, 2012; Novelle *et al.*, 2013; Aydin, 2014; Boström *et al.*, 2014).

De acordo com os estudos realizados até o momento a irisina parece possuir potencial termogênico e pode estar envolvida no metabolismo da glicose, o que sugere um potencial terapêutico na obesidade e no diabetes tipo 2, ambos fatores associados com a síndrome metabólica (Erickson, 2013; Novelle *et al.*, 2013; Irving *et al.*, 2014).

A síndrome dos ovários policísticos (PCOS) é o distúrbio endocrinológico mais frequente em mulheres com idade reprodutiva e principal causa de infertilidade (Balen e Rajkowha, 2003; Orio *et al.*, 2003; Spritzer, 2014; Spritzer *et al.*, 2015), afetando cerca de 6% a 19% desta população, dependendo do critério diagnóstico (March *et al.*, 2010; Mario *et al.*, 2017). De acordo com os critérios de Rotterdam, o diagnostico da PCOS se caracteriza como a presença de pelo menos duas das seguintes manifestações: hiperandrogenismo clínico ou bioquímico, anovulação ou oligoovulação, e ovários policísticos ao ultrassom (Spritzer *et al.*, 2000; Group, 2004; Messinis *et al.*, 2014; Aliasghari *et al.*, 2017). As mulheres com PCOS frequentemente apresentam obesidade, com acúmulo de gordura na região visceral (obesidade

central) (Balen e Rajkowha, 2003; Orio et al., 2003; Messinis et al., 2014; (Mario *et al.*, 2017). Em consequência, observam-se alterações do metabolismo lipídico e maior suscetibilidade para hipertensão arterial, tolerância diminuída à glicose e diabete tipo 2, todos constituindo-se em fatores de risco para doença cardiovascular (Balen e Rajkowha, 2003; Orio et al., 2003; Spritzer, 2014). Os fenótipos mais prevalentes da PCOS são, a PCOS clássica (c-PCOS) caracterizada pela presença de oligo/amenorreia e/ou ciclos anovulatórios associados ao hiperandrogenismo clínico ou bioquímico e com ou sem ovários policísticos ao ultrassom e o fenótipo ovulatório, (ov-PCOS), definido pela presença de hiperandrogenismo e ovários policísticos em mulheres com ciclos ovulatórios (Mario *et al.*, 2017).

O estudo de novas alternativas farmacológicas para tratamento dos distúrbios metabólicos relacionados com a PCOS têm sido alvo de pesquisas em todo mundo ao longo dos anos. A irisina surgiu recentemente como um potencial protagonista para o tratamento destas alterações metabólicas relacionadas com a PCOS, em especial a obesidade e a resistência à insulina.

Os estudos que avaliaram associações entre PCOS e níveis de irisina são escassos na literatura e apresentaram resultados ainda não conclusivos. Chang et al. observaram níveis séricos de irisina maiores em mulheres com PCOS, com ou sem síndrome metabólica, sugerindo que a irisina possa ser considerada um fator de risco para PCOS (Chang *et al.*, 2014). Este resultado foi confirmado em estudo recente (Adamska *et al.*, 2016). Por outro lado, outros trabalhos não confirmam este achado (Li *et al.*, 2015; Gao *et al.*, 2016) e um dos estudos descreve níveis significativamente maiores de irisina em mulheres com sobrepeso/obesidade, independente de PCOS (Li et al., 2015). Este estudo também demonstrou que o tratamento com metformina, em pacientes diabéticas com PCOS, durante 6 meses, resultou em diminuição significativa dos níveis de irisina (Li et al., 2015).

Quanto às relações entre irisina e variáveis antropométricas e de composição corporal na PCOS, Li H. et al. estudando uma amostra de casos e controles, observaram uma correlação positiva entre irisina e índice de androgênios livres (IAL), índice de massa corporal (IMC), relação cintura/quadril (RCQ), percentual de gordura total, triglicerídeos e marcadores de resistência insulínica, incluindo o clamp euglicêmico hiperinsulinêmico (Li *et al.*, 2016). Adamska et al. encontraram concentrações de irisina sérica basal após 120 minutos do clamp euglicêmico hiperinsulinêmico foram mais elevadas em mulheres com PCOS do que em controles. Observaram ainda que durante a realização do clamp a infusão de insulina resultou numa diminuição da concentração de irisina apenas no grupo PCOS (Adamska et al., 2016).

No entanto, no que se refere à determinação dos níveis circulantes de irisina, foram testados de forma comparativa dois kits ELISA: CUSABIO (Catalogo No. CSB-EQ027943HU) e Phoenix Pharmaceuticals (Catalogo No. EK-067-29) e não foi encontrada diferença significativa entre os resultados, sugerindo que os kits testados são adequados para mensuração dos níveis de irisina (Li et al., 2015).

Enfim, os dados disponíveis até agora relacionando irisina e PCOS não são conclusivos, sendo indispensável a continuidade das pesquisas. Sendo assim, torna-se relevante aprofundar o conhecimento sobre aspectos ainda controversos nesta área, como a relação de irisina com obesidade, composição corporal e o estado metabólico. Por tal motivo, a avaliação de possíveis associações entre níveis séricos de irisina e massa magra e gorda corporais, bem como com variáveis metabólicas e hormonais poderá contribuir para um melhor entendimento de alterações metabólicas relacionadas com PCOS – uma condição complexa que atinge uma parcela significativa de mulheres em idade reprodutiva.

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Circulating levels of irisin in Polycystic Ovary Syndrome and control women: a case-control study

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ABSTRACT

Introduction: Polycystic ovary syndrome (PCOS) has been recognized as a metabolic disorder, manifested by abdominal obesity, insulin resistance, dyslipidemia and hypertension. Irisin, a newly adipokine/miokine, seems to mediate thermogenesis and metabolic homeostasis.

Objectives: To determine circulating levels of irisin in women with PCOS and non-hirsute, ovulatory control women, and to evaluate the association between irisin features with hormonal and metabolic variables, and with body composition in the PCOS and control groups.

Methods: This case-control study was carried out with 49 PCOS and 33 non-hirsute ovulatory women with similar age and body mass index (BMI). Dual-energy X-ray Absorptiometry (DXA) evaluated body composition. Irisin serum levels were measured by a human ELISA kit.

Results: Systolic blood pressure, HOMA, total testosterone and FAI were significantly higher in PCOS, while SHBG was lower in the patients group. After BMI-stratification, fat mass and fat mass/lean mass ratio were lower in normal-weight women than in overweight/obese women. Normal-weight PCOS showed less total lean mass than overweight/obese PCOS and control subgroups. Appendicular lean mass/BMI ratio was significantly higher in normal-weight controls than in overweight/obese control woman, but PCOS subgroups were similar to normal-weight and overweight/obese controls. Irisin serum levels were significantly higher in overweight/obese patients with PCOS than in normal-weight controls. Circulating irisin correlated positively with total lean mass and fat mass/lean mass ratio in women with PCOS, independently of androgen excess.

Conclusion: Our data suggest an association of irisin with body composition.

Key words: irisin, insulin resistance, PCOS, DXA.

INTRODUCTION

Polycystic Ovary Syndrome (PCOS), a prevalent endocrine disorder, affects 6 to 19% of women of reproductive age, depending on diagnostic criteria (March *et al.*, 2010; Yildiz *et al.*, 2012). PCOS has also been recognized as a metabolic disorder, manifested by abdominal obesity, insulin resistance, dyslipidemia and hypertension. These factors seem to increase the risk of cardiovascular disease and metabolic syndrome in this population (Apridonidze *et al.*, 2005; Ehrmann *et al.*, 2006; Spritzer e Wiltgen, 2007; Wiltgen e Spritzer, 2010; Diamanti-Kandarakis *et al.*, 2012; Spritzer *et al.*, 2015).

To date, a number of new proteins have been proposed as surrogate markers of insulin resistance (Polak *et al.*, 2017). Irisin, a newly adipokine/myokine (Boström *et al.*, 2012; Moreno-Navarrete *et al.*, 2013; Boström *et al.*, 2014; Panati *et al.*, 2016), results from cleavage of the extracellular portion of the Fibronectin Type III Domain Containing 5 (FNDC5) protein in response to physical activity (Panati *et al.*, 2016). Irisin has been implicated in the differentiation of white adipocytes into brown fat, mediating thermogenesis and beneficial effects on metabolic homeostasis (Boström *et al.*, 2012; Erickson, 2013; Novelle *et al.*, 2013; Boström *et al.*, 2014; Irving *et al.*, 2014; Polak *et al.*, 2017).

The relationship between circulating irisin and PCOS features is still unclear. Some studies have shown that elevated serum irisin levels are associated with hyperandrogenism (Li *et al.*, 2016) and insulin resistance (Chang *et al.*, 2014; Bostancı *et al.*, 2015; Li *et al.*, 2015; Adamska *et al.*, 2016; Li *et al.*, 2016), while others have been reported decreased (Abali *et al.*, 2016) or unchanged (Gao *et al.*, 2016) irisin levels in PCOS. Therefore, our aim was to determine circulating levels of irisin in women with PCOS and nonhirsute, ovulatory control women, and to evaluated the association between irisin features with hormonal and metabolic variables, and with body composition in the PCOS and control groups.

MATERIAL AND METHODS

Patients and Controls

This case–control study was carried out with women of reproductive age seen at the Gynecological Endocrinology Unit at Hospital de Clínicas de Porto Alegre (HCPA), Brazil. Women with a body mass index (BMI) ranging from 18.0 to 39.9 kg/m^2 were selected for the study. Forty-nine hirsute oligo-amenorrheic women (≤9 cycles/year), presenting with increased levels of serum testosterone (>0.8 ng/mL) and/or free androgen index (FAI) (>6.1) and/or polycystic ovaries in the absence of other disorders causing hirsutism (Group, 2004; Azziz *et al.*, 2009), were included as PCOS. A control group was set up with 33 non-hirsute ovulatory women (regular cycles and luteal phase progesterone levels higher than 3.8 ng/mL) with normal androgen levels and normal ovaries on ultrasound, recruited through public advertisement at the same Gynecological Endocrinology Unit. All participants had to be at least 2 years after menarche. None of the women from PCOS and control group had received oral contraceptives, insulin sensitizers or antiandrogens for at least 3 months before the study. Women with diabetes, renal or liver disease, thyroid dysfunction or pregnancy were also excluded. The Research and PostGraduate Group of Hospital de Clinicas de Porto Alegre (GPPG/HCPA) and the local Ethics Committee approved the study protocol, and written informed consent was obtained from all participants.

Study protocol

Medical interview and physical examination were performed as previously described (Wiltgen *et al.*, 2009; Santos *et al.*, 2017). Hirsutism was defined as a modified Ferriman-Gallwey score ≥8 (Koppen e Kalkhoven, 2010). Blood pressure (BP) was measured after a 10-minute rest (Lo e Sun, 2013). Anthropometric measurements included body weight, height, BMI and waist circumference (Barroso *et al.*, 1999; Vázquez-Vela *et al.*, 2008).

Resting metabolic rate was obtained by indirect calorimetry (FITMATE GS, Rome, Italy) (Toscani *et al.*, 2011). Total lean mass and fat mass was evaluated by Dual-energy X-ray Absorptiometry (DXA) (GE Medical Systems Lunar, Connecticut, USA) as reported before by our group (Mario *et al.*, 2012). Appendicular lean mass was calculated by the sum of the lean mass of arms and legs divided by the height squared (m). Habitual physical activity was measured using a digital pedometer (BP 148 Techline, São Paulo, Brazil) as previously described (Graff *et al.*, 2012; Mario *et al.*, 2017). Hormonal and metabolic assessment, as well as transvaginal/transabdominal ultrasound, were performed between the 2nd and 8th of the menstrual cycle or on any day if the patient was amenorrheic (Pardo *et al.*, 2014). Blood samples were drawn between 8 AM and 10 AM, after an overnight 12-hour fast, for determination of serum irisin, and lipid profile at baseline and glucose and insulin before and 2 hours after ingestion of 75 g oral anhydrous glucose (oral glucose tolerance test). Blood samples were also assessed for measurement of sex hormone–binding globulin (SHBG), and total testosterone (TT). Free androgen index was estimated by dividing TT (nmol/l) by SHBG (nmol/l) x 100. Homeostasis model assessment index to estimate insulin resistance (HOMA) was calculated by multiplying insulin (mIU/ml) by glucose (mmol/l) and dividing this product by 22.5 (Crujeiras *et al.*, 2014). Low-density lipoprotein cholesterol was estimated indirectly with the Friedewald formula (Friedewald *et al.*, 1972).

Biochemical and hormonal assays

Total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, and glucose were determined by colorimetric-enzymatic methods (Bayer 1800 Advia System, Mannheim, Germany). Insulin (sensitivity (S) of 0.2 mIU/ml), TT (S = 0.1 nmol/l) and SHBG (S = 0.02 nmol/l) were measured with chemiluminescence immunoassays (Siemens Centaur XP and Immulite 2000 Siemens, Deerfield, USA), with intra-assay coefficient of variation

(CV) of 2.0%, 3.3% and 5.3%, and interassay CV of 4.3%, 7.5% and 6.6%, respectively. Irisin serum levels were determined using a human enzyme-linked immunosorbent assay (ELISA) kit with a detection limit of 3.12 ng/ml (CUSABIO Biotech Co. Ltd., Maryland, USA; Cat. No: CSB- EQ027943HU), and intra- and interassay CV <10%. Analyses were performed in accordance with the manufacturer's instructions.

Statistical analysis

Data were described as mean ± SD or median and interquartile range (25% – 75%). Comparisons between means and medians were analyzed by the unpaired two-tailed Student's *t*-test or Mann-Whitney *U* test, respectively. Group means were compared by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Pearson's or Spearman's rank correlation coefficients were calculated between variables using a two-tailed test for significance, followed by adjustement by FAI (linear regression). Log10 transformation was used to normalize the distribution of non-Gaussian variables, and mean values were back-transformed for presentation. Data were considered statistically significant at $p<.05$. The Statistical Package for the Social Sciences 19 (SPSS, Chicago, IL) was used in the analysis.

RESULTS

Clinical, metabolic and hormonal features for control women and patients with PCOS are presented in Table 1. Most participants were white, and the remaining women were of mixed African and European ancestry. BMI, waist circumference, diastolic BP, fasting and 2h glucose, and lipid profile were similar between the groups. As expected, systolic BP, HOMA, TT and FAI were higher in PCOS ($p\leq0.045$), while SHBG was lower in patients with the syndrome ($p=0.015$).

Table 2 shows body composition and habitual physical activity data for control women and patients with PCOS, stratified according to BMI (<25 or ≥25 kg/m²). Resting metabolic rate and steps per day of the participants did not differ between the studied groups. Fat mass and fat mass/lean mass ratio were lower in normal-weight women than in overweight/obese women, regardless of PCOS condition ($p \leq 0.049$). Normal-weight PCOS showed less total lean mass than overweight/obese PCOS and control subgroups, but normal-weight controls were similar to normal-weight PCOS ($p=0.049$). Appendicular lean mass/BMI ratio was significantly higher in normal-weight controls than in overweight/obese control woman, but PCOS subgroups were similar to control both subgroups ($p=0.025$).

Circulating irisin levels were similar between PCOS and controls 425.0 (227.5 – 698.9) vs. 378.8 (196.3 – 661.2); ng/ml; $p = 0.699$]. In turn, subgroups analysis indicated that irisin serum levels were significantly higher in overweight/obese patients with PCOS than in normal-weight controls [579.9 (292.8 – 793.9) vs. 138.2 (97.6 – 293.7) ng/ml; $p=0.047$] (Fig. 1). In addition, in women with PCOS, serum irisin correlated positively with total lean mass and fat mass/lean mass ratio ($r = 0.405$ and $r = 0.437$, respectively; $p \leq 0.004$), even after FAI adjustment ($p \leq 0.009$) (Table 3).

DISCUSSION

In the present study, irisin serum levels were significantly higher in overweight/obese women with PCOS in comparison with normal-weight controls. In addition, positive correlations were found between irisin concentrations and total lean mass as well as fat mass/lean mass ratio, independently of androgens in PCOS. These results support the notion that altered lean and fat mass distribution may be linked to irisin levels in PCOS women. Previous studies have also reported an association between circulating irisin and body composition parameters (Li et al., 2015; Pukajło et al., 2015; Gao et al., 2016).

Recent studies from Taiwanese (Chang et al., 2014), Chinese (Li et al., 2015; Li et al., 2016), Turkish (Bostancı et al., 2015) and Polish (Adamska et al., 2016) populations have shown higher serum irisin concentrations in PCOS patients in comparison to healthy control women. Chang et al. (Chang et al., 2014) demonstrated elevated irisin serum levels in normal-weight PCOS patients when compared to matched controls. Li et al. (Li et al., 2015) showed that irisin concentrations were significantly higher in overweight/obese controls and PCOS than in normal-weight women. These authors also observed a significant decrease in irisin levels associated with improvement on insulin sensitivity in PCOS women after metformin therapy. Increased irisin levels were also reported in PCOS women with high FAI (Li et al., 2016), suggesting that circulating irisin might be a primary predictor of androgen excess, insulin resistance and metabolic syndrome in PCOS. In addition, the association between circulating irisin and insulin resistance in PCOS has been often reported (Chang et al., 2014; Bostancı et al., 2015; Li et al., 2015; Adamska et al., 2016). However, while elevated irisin levels could be a compensatory mechanism to ameliorate metabolic conditions in insulin resistant subjects, this hypothesis is still not consensual, as other authors found contrasting results. In this sense, Abali et al. (Abali et al., 2016) reported lower serum irisin concentrations in women with PCOS compared to BMI and age-matched Turkish controls and Gao et al. (Gao *et al.*, 2016) showed similar irisin levels in Chinese PCOS and control women, even when participants were stratified by BMI. Considering genetics and lifestyle differences between Asian and Caucasian populations, further studies with PCOS women from different ethnicities are needed in order to clarify this issue.

In fact, in the present study, serum irisin seems to be more related to BMI and body composition than specifically to PCOS. The androgen-independent association between serum irisin with total lean mass and fat mass/lean mass ratio suggests body content and distribution is a relevant factor associated with metabolic dysfunction in PCOS. Our results are in

agreement with other reports, showing a positive correlation between total lean mass and irisin concentrations in PCOS (Gao *et al.*, 2016). In that study, irisin presented a day-night rhythm, its circulating levels were not affected by intake of a standardized meal and were not associated with caloric intake or diet quality, and as well, irisin levels were increased at the end of exercise. Also, a positive association between irisin concentrations and android fat distribution was found in PCOS versus aged-matched control women (Pukajło et al., 2015).

We measured irisin serum levels with a human ELISA kit commercially available in our country. As specifyed by the manufacturer, the assay has high sensitivity and excellent specificity for detection of human irisin without significant cross-reactivity or interference between human irisin and analogues. We were aware about analytical variability from different irisin ELISA kits. However, the kit used in our study has been previously compared to another very common one by Li et al. (Li *et al.*, 2015). These authors showed that both irisin ELISA kits were appropriate for the analysis of clinical samples, including those from PCOS.

One limitation of this study was the relatively small sample size, which precluded additional statistical analysis. However, to our knowledge, this is the first study characterizing irisin serum levels in women with PCOS from southern Brazil. A strength of our study was the use of DXA to assess body composition. DXA is considered the gold standard method to directly assess body compartments without inferring data from the measurement of only one compartment. Moreover, the exam has an excellent accuracy and great reproducibility.

In conclusion, irisin concentrations were not directly related to PCOS in a sample of women from southern Brazil. Our data suggest an association of irisin with body composition.

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Legend of Figure 1: Serum irisin in PCOS and control women, stratified according to BMI (kg/m^2). The number of serum samples tested per group appears within parentheses. Values are expressed as median and interquartile range (25%–75%). One-way ANOVA plus Tukey post hoc test.

Figure 1

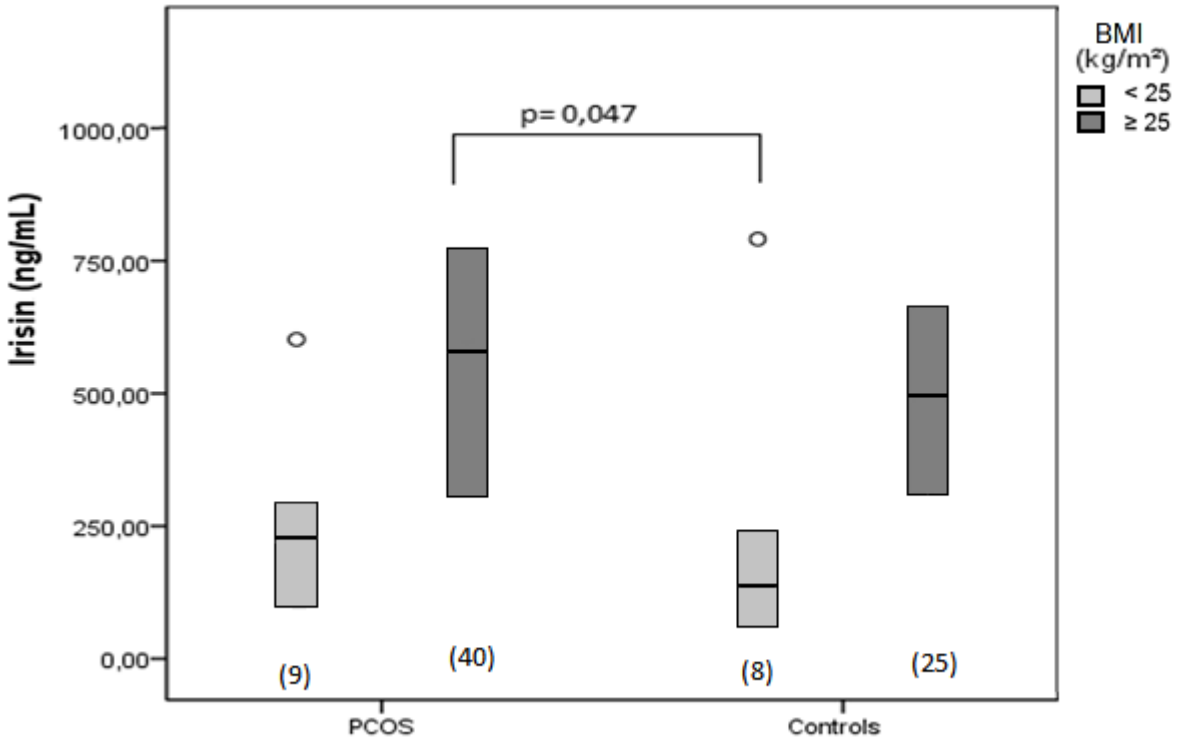

Table 1: Clinical, metabolic and hormonal features for control women and patients with PCOS

Characteristic	Control (n=33)	PCOS (n=49)	p
Age (years)	28.0 (20.0 – 31.0)	23.0 (19.0 – 29.0)	0.095
BMI (kg/m²)	28.7 ± 4.3	29.2 ± 5.7	0.593
Waist circumference (cm)	85.3 ± 10.0	86.6 ± 13.5	0.647
Systolic BP (mmHg)	110.0 (110.0 – 120.0)	120.0 (110.0 – 125.0)	**0.045**
Diastolic BP (mmHg)	74.0 (70.0 – 80.0)	80.0 (70.0 – 80.0)	0.109
Fasting glucose (mg/dl)	85.0 (81.0 – 89.0)	84.5 (81.2 – 91.5)	0.760
Glucose 2h (mg/dl)	97.0 (85.0 – 109.0)	94.0 (81.0 – 116.0)	0.919
HOMA	2.3 (1.5 – 2.9)	3.4 (1.8 – 4.6)	**0.039**
Triglycerides (mg/dl)	84.5 (65.2 – 113.7)	93.5 (63.0 – 134.0)	0.461
Total cholesterol (mg/dl)	177.7 ± 31.1	176.2 ± 33.5	0.835
HDL cholesterol (mg/dl)	48.4 ± 9.6	46.8 ± 12.9	0.561
LDL cholesterol (mg/dl)	109.1 ± 24.2	108.0 ± 26.2	0.853
Total testosterone (ng/ml)	0.5 ± 0.2	0.7 ± 0.2	**<0.001**
SHBG (nmol/l)	34.9 (29.0 – 48.8)	27.8 (14.5 – 41.7)	**0.015**
FAI	5.0 (3.6 – 7.6)	7.7 (5.1 – 15.7)	**0.001**

Values are expressed as mean ± SD (Student's t-test) or median and interquartile range (25%–75%) (Mann-Whitney U test). BMI = body mass index; BP = blood pressure; FAI = free androgen index; HDL = high-density lipoprotein; HOMA = homeostasis model assessment index; LDL = low-density lipoprotein; PCOS = Polycystic Ovary Syndrome; SHBG = sex hormone–binding globulin.

Table 2: Body composition and habitual physical activity for control women and patients with PCOS, stratified according to BMI (kg/m^2)

Characteristic	BMI <25		BMI ≥25		p
	Control (n=8)	PCOS (n=9)	Control (n=25)	PCOS (n=40)	
FM (kg)	22.5 ± 4.3^{a}	21.8 ± 6.5^{a}	35.5 ± 7.8^{b}	34.7 ± 11.3^{b}	**0.049**
LM (kg)	36.2 ± 3.7^{ac}	34.3 ± 3.2^{a}	39.8 ± 4.6^{bc}	42.1 ± 6.3^{b}	**0.049**
Appendicular LM/BMI (m^{-2})	704.1 ± 77.7^{a}	675.2 ± 90.2^{ab}	590.8 ± 99.3^{b}	619.0 ± 99.1^{ab}	**0.025**
FM/LM	0.6 ± 0.1^{a}	0.6 ± 0.2^{a}	0.9 ± 0.2^{b}	0.8 ± 0.2^{b}	**0.027**
RMR (kcal/day)	1383 ± 246	1302 ± 206	1484 ± 214	1477 ± 207	0.129
Steps/day	6732 (5734-8388)	5772 (3558-8996)	5634 (3398-7432)	5485 (3808-6887)	0.740

Values are expressed as mean ± SD or median and interquartile range (25%–75%). ^{abc} Different letters indicate statistical difference by one-way ANOVA plus Tukey post hoc test. BMI = body mass index; FM = fat mass; LM = total lean mass; PCOS = Polycystic Ovary Syndrome; RMR = resting metabolic rate.

Table 3: Serum irisin *vs.* significantly correlated variables for control women and patients with PCOS

Variable	Control (n=33)		PCOS (n=49)	
		Adjustment for FAI*		Adjustment for FAI*
LM	r = 0,087; p = 0,628	r = 0,072; p = 0,706	r = 0,405; p = **0,004**	r = 0,376; p = **0,009**
FM/LM	r = 0,200; p = 0,265	r = 0,148; p =0,444	r = 0,437; p = **0,001**	r = 0,437; p = **0,002**
HOMA	r = 0,352; p = **0,048**	r = 0,253; p = 0,186	r = 0,319; p = **0,027**	r =0,256; p = 0,083

Pearson's correlation coeficients. *Linear regression. FAI = free androgen index; FM = fat mass; HOMA = homeostasis model assessment index; LM = total lean mass; PCOS = Polycystic Ovary Syndrome.