The RELATION OF SERUM IRISIN LEVEL WITH METABOLIC AND HORMONAL CHANGES IN RAT MODEL OF POLYCYSTIC OVARY

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ABSTRACT

Background: Polycystic ovary syndrome (PCOS) is one of the leading causes of infertility in female. It is usually associated with insulin resistance (IR), obesity, hyperlipidemia, and type II diabetes. Irisin is amyokine which increases energy expenditure and protects against insulin resistance and obesity. There were controversial studies about the levels of irisin and its relation to hormonal and metabolic changes in PCOS. This study was designed to estimate serum level of irisin in letrozole-induced PCOS in both lean and obese female rats, and to study the link between serum irisin level and some metabolic and hormonal parameters in PCOS.

Material and Methods: This study was conducted on 48 young virgin female of local strain albino rats. They were randomly divided into 2 equal groups: Group I: lean group were fed on normal laboratory chow diet and Group II: A high fat diet induced obesity group and each group was subdivided into group A (control) and group B (letrozole induced PCOS). Fasting serum irisin, Lutenizig hormone (LH), Folliclular stimulating hormone (FSH), testosterone, estradiol, progesterone, insulin, glucose, triglycerides, cholesterol, LDL and HDL levels were estimated. In addition, body mass index (BMI) and Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) were calculated in all groups at the end of the experiment.

Results: Serum irisin level was elevated in both lean and obese PCOS groups when compared to both lean and obese control groups. In addition, obese PCOS group had significant higher level of irisin when compared to lean PCOS group.

Conclusion: Serum irisin level was positively correlated with BMI, HDL and testosterone levels and negatively correlated with HOMA- IR, insulin, cholesterol, triglycerides and LDL levels. Irisin may have a role in the development of polycystic ovary syndrome.

Key words: Irisin – PCOS- lean – obese.

INTRODUCTION

Irisin is a newly discovered exercise-mediated myokine secreted by skeletal muscle and play an important role in regulation of energy metabolism by inducing browning of white adipose tissue [1]. Irisin is also released by adipocytes [2]. The regulation of irisin as well as its role in glucose metabolism remains to be clarified [2, 3].

Irisin is also related to increased incidence of metabolic syndrome and cardiovascular disease in humans, and its secretion may be increased as a compensatory mechanism to overcome an underlying irisin resistance in these subjects [4].

PCOS is a hormonal disorder common among women of reproductive age.[5]. It is characterized by hyperandrogenism, ovulatory dysfunction and infertility [6].

Also, it is associated with insulin resistance, obesity, hyperlipidemia, increased prevalence of type II diabetes, endothelial dysfunction and oxidative stress [7, 8].

Few researchers illustrate the relationship between serum irisin level and PCOS patients [9, 10]. So, this present study tries to elucidate this relationship, by estimating the level of serum irisin in lean and obese PCOS rats and also, by searching for the link between serum irisin level and BMI, IR, and lipid profile in both lean and obese PCOS rats.

MATERIAL AND METHODS

This study was carried out on 48 young virgin female of local strain albino rats of 6 weeks old weighing 90-100 gm. They were obtained from the animal house faculty of veterinary medicine Zagazig University.

The rats were kept in steel wire cages (6/cages) in the animal house of the faculty of medicine Zagazig University under hygienic conditions.

The rats had free access to water and chow, were kept at room temperature and were maintained on a 12 h light/ dark cycle. The experimental protocol was approved by physiology department and by local medical

ethics committee in faculty of medicine of Zagazig University (Institutional Review Board, IRB).

The animals were randomly divided into 2 main groups (n= 24): Group I : lean rats, fed on commercial rat standard chow consisted of 25.8% protein, 62.8% carbohydrates and 11.4% fat Group II: high fat diet induced obese rats "HFD" rats fed on high fat diet generally contain protein 20%, carbohydrates 35% and fat 45%, mainly in form of lard and soy bean for 9 weeks[11]. Then each group further subdivided into subgroup A (control) given 1ml water orally by gavage daily for 21 days, subgroup B (letrozole induced PCOS): oral administration of letrozole (non-steroidal aromatase inhibitor, ACDIMA international) (Daily 0.5 mg/kg dissolved in water by gavage) for 21 consecutive day[12].

Determination of Sexual cycle: Smears were obtained daily by vaginal washing with saline. The fresh unstained samples were evaluated microscopically during the treatment period [12]. Cycles with duration of 4 to 5 days were considered regular estrus phases were determined according to Marcondes et al. [13] and Goldman et al. [14] by examining the vaginal smear into:

- The proestrus phase: the vaginal smear consists of a predominance of nucleated epithelial cells with smooth margins.

-The estrus phase: the vaginal smear shows large anucleated cornified (keratinized) cells with irregular margins.

-The met estrus phase: the vaginal smear shows many cornified cells plus infiltration of leukocytes.

- The diestrus phase: the vaginal smear shows absence of the cornified cells and presence of small leukocytes.

The observation of cornified cells in the smears during a minimum of 10 consecutive days was defined as persistent estrous, indicating anovulation and development of follicular cysts.

Calculation of BMI: 24 h after the end of the study (after the last dose of letrozole), and after overnight fasting, rats weighed and BMI were calculated according to Novelli et al. [15] from the following equation:

BMI =body weight (gm)

length^2 (cm^2) (nose to anus)

Collection of blood and tissue samples: Rats were anaesthetized by ether inhalation, blood samples were collected from orbital sinus (sampling of controls taken in the estrus phase) and ovaries were dissected and immediately fixed in 4% paraformaldehyde. Blood centrifuged at 3000 rpm for 15 minutes, the supernatant serum was stored at -20°C [16] until used for:

Estimation of serum irisin level, according to Bostram et al. [1] using rat irisin ELISA rat kit (Catalog # K4761-100), biovision, Milpitas Blvd., Milpitas, CA 95035 USA was performed. Estimation of serum LH, FSH, estradiol, progesterone and testosterone levels: according to Tietz [17] .using rat kits: BC-1031, BC-1029, BC-1111, BC-1113 and BC-1115, respectively, BioCheckInc 323 Vintage Park Dr. Foster City, CA – 94404.

Estimation of serum glucose level according to Tietz [17] and serum insulin level by enzyme– linked immunosorbent assay (ELISA) according to Temple et al. [18]. Kits for estimation of serum glucose and insulin levels were purchased from (Biosource Europe S.A.Belgium).

Measurement of homeostasis model assessment (HOMA-IR) index was calculated according to Sun et al. [19] as follows:

[HOMA-IR] = fasting serum glucose (mg/dl) × fasting serum insulin (μIU/ml)/405.

Estimation of lipid profile as follows: Total serum cholesterol level: according to Tietz [17] [serum TG level: according to Fossati [20] , serum HDL levels according to Nauck et al. [21] and serum LDL levels was calculated according to Friedewald et al. [22] as follows: LDL = TC -HDL – TG/5(Kits for estimation of serum glucose, insulin, cholesterol, TG and HDL levels were purchased from Biosource Europe S.A.Belgium).

Histopathological examination: The abdominal cavities of the rats were opened. Ovaries were dissected and fixed in 10% buffered formalin for 6 hours at room temperature and washed in a phosphate buffer saline solution. For light microscopy, fixed tissues were dehydrated in an ascending series of ethanol, cleared in xylene and embedded in paraffin. 5 mm thick sections were mounted in slides previously treated with 3-

aminopyropyl triethoxysilane and stained with hematoxylin-eoisin preliminary observation [23]. The pathologist was blinded to the treatment.

Statistical analysis: The data obtained in the present study were expressed as mean ±SD for quantitative variables and statistically analyzed according to the methods described by Kirkwood [24]. The statistical analysis is done by using SPSS program (19) (SPSS Inc. Chicago, IL, USA).

ANOVA (Post hoc) test was used to compare means among more than two groups.

P value < 0.05 was considered statistically significant.

*Corrélation coefficient (r):*Pearson's correlation analysis was performed to illustrate the relationships between serum irisin and the studied metabolic parameters among different groups. Pearson's correlation was considered significant at P values <0.05.

RESULTS

Histopathological findings

Ovaries from the control groups (IA and IIA groups) histologically (under 200_ magnification) had numerous variable sized Graffian follicles at different stages of maturation surrounded by dense spindle shaped ovarian stroma **(figure 1 &2).**

In ovaries from PCO rats (IB and IIB groups) histologically (under 200 magnification), it showed numerous cystically dilated follicles surrounded by dense ovarian stroma **(Figure 3 & 4).**

This study revealed that in lean PCOS group (IB) there were significant high levels of serum irisin, LH, testosterone and BMI when compared with that of lean control group (IA), (P value: < 0.001,<0.001,<0.001,< 0.05 respectively). While there were significant low levels of serum estradiol and progesterone in group (IB) compared to control group (P value: < 0.001, <0.001 respectively), however, no significant change in serum FSH, insulin, glucose, calculated HOMA, serum cholesterol, triglycerides, LDL and HDL levels, (P value: > 0.05).

Moreover, in obese PCOS group (IIB) there were significant high levels of serum irisin, LH, testosterone, insulin, cholesterol, triglycerides , LDL levels, BMI and calculated HOMA-IR compared with that of obese control Group (IIA), (P value: <0.001,<0.001,<0.001,<0.001,< 0.001,<0.001,<0.001,<0.001,<0.001 respectively).While there were significant low levels of serum estradiol ,progesterone and HDL compared with that of obese control,(P value: < 0.001, < 0.001, < 0.001 respectively), however, no significant change in serum FSH and glucose, (P > 0.05).

Also, obese group (IIA) showed significant high levels of serum irisin, glucose, insulin, cholesterol, triglycerides, LDL levels, BMI and calculated HOMA-IR compared with that of lean control (IA) (P value: < 0.001, < 0.001, < 0.001< 0.001, < 0.001, < 0.001, < 0.001,< 0.001 respectively and significant low levels of serum HDL (P value: < 0.001). However there were no significant change in serum levels of LH, FSH, testosterone, estradiol and progesterone between the two groups (P > 0.05).

In addition, obese PCOS group (IIB) showed significant high levels serum irisin, LH, glucose, insulin, cholesterol, triglycerides, LDL levels, BMI and calculated HOMA-IR compared with that of lean control (IB) (P value: < 0.001, <0.05, <0.001,<0.001,< 0.001,< 0.001, < 0.001,<0.001, respectively) and significant low levels of serum HDL (P value: < 0.001). However there were no significant change in serum levels of FSH, testosterone, estradiol and progesterone between the two groups (P >0.05) **(Table 1; Figures 5-7).**

Irisin showed significant positive correlation with BMI, testosterone and HDL levels in (Group IB: r = 0.779** p <0.01, r= 0.921*** p <0.001, r= 0.879*** p <0.001 respectively),(Group IIA: r= 0.753** p <0.01, r= 0.879*** p <0.001, r= 0.781** p <0.01 respectively) , (Group IIB: r = 0.779^{**} p <0.01, r = 0.921^{***} p <0.001, r= 0.867^{**} p <0.001 respectively) , while irisin showed significant negative correlation with insulin, HOMA IR, cholesterol, triglycerides and LDL levels in (Group IB: r= -0.885*** p <0.001, r= -0.912*** p <0.001, r= -0.724*** p <0.01, r=-0.905*** p <0.001, r= -0.902*** p <0.001 respectively), (Group IIA: r= -0.871*** p <0.001, r= -0.888*** p <0.001, r= -0.754** p <0.01, r= -0.776** p <0.01, r= -0.680* p

<0.05 respectively), (Group IIB: r= -0.885*** p <0.001, r= -0.818*** p <0.001, r= -0.724** p <0.01, r= -0.905*** p <0.001, r= -0.902*** p <0.001 respectively). However, no significant correlation between irisin, LH, FSH, estradiol, progesterone and glucose levels in those groups (P > 0.05).

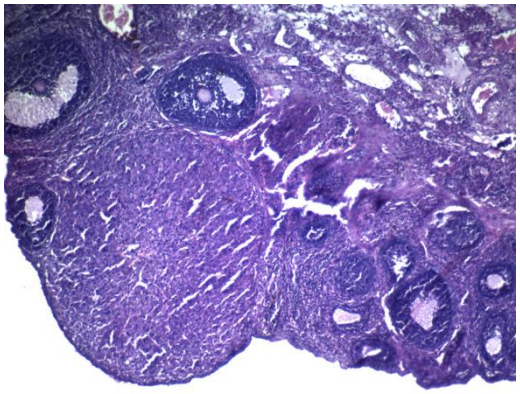

Figure 1: Histologic ovarian tissue sections dyed with H&E in group IA (lean control) under 200_ magnification.

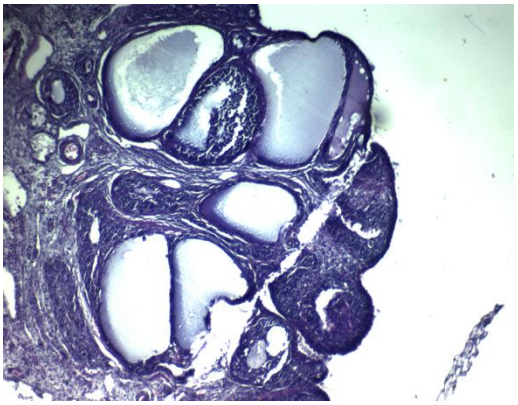

Figure 2: Histologic ovarian tissue sections dyed with H&E in group IB (lean PCOS) under 200_ magnification.

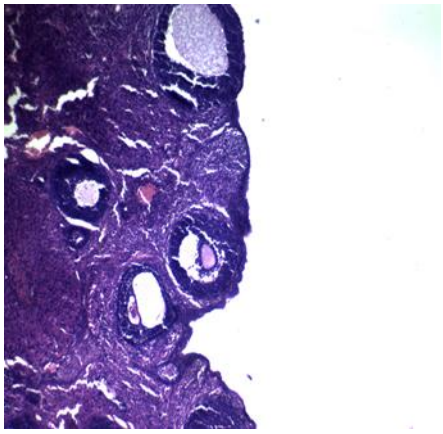

Figure 3: Histologic ovarian tissue sections dyed with H&E in group IIA (obese control) under 200_ magnification.

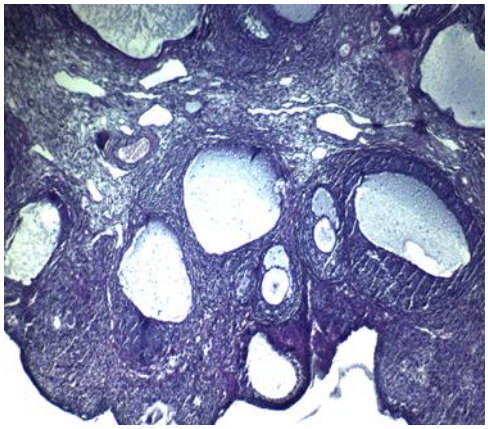

Figure 4: Histologic ovarian tissue sections dyed with H&E in group IIB (obese PCOS) under 100_ magnification.

Table 1: **Shows all measured parameters in all studied groups expressed as (mean±SD):**

groups / parameters	Group IA	Group IB	Group IIA	Group IIB
irisin(ng\ml)	7.57± 1	11.16± 0.98^{***a}	15.53± 1.3^{***a,b}	17.7± 1.5^{***a,b,c}
BMI (gm/cm2)	0.48± 0 .028	0.53± 0.031^{*a}	0.83± 0.038^{***a,b}	0.89± 0.052^{***a,b,c}
LH (IU/ml)	2.16± 0.29	5.96± 0.76^{***a}	2.31± 0.29	6.5± 0.84^{***a,c,*b}
FSH (IU/ml)	3.76± 0.31	3.82± 0.34	3.76± 0.32	3.98± 0.22
estradiol (pg/ml)	31.4± 4.26	14.2± 2.18^{***a}	32.03±4.2	13.69± 2.3^{***a,c}
Progesterone (pg/ml)	7.7 ± 0.73	5.59± 0.96^{***a}	7.81± 0.97	5.02± 0.99 ^{***a,c}
Testosterone (pg/ml)	77.75 ± 10.4	233.7 ± 12.6^{***a}	80.6 ± 4.5	235.3 ± 12.5^{***a,c}
Insulin (µIU/ml)	12.35± 1.55	13.29± 1.45	22.5 ± 1.2^{***a,b}	26.25± 2.23^{***a,b,c}
Glucose (mg/ dl)	84.6± 6.2	85± 5.6	140.8± 6.3^{***a,b}	143.75± 4.3^{***a,b}
HOMA-IR	2.58± 0.39	2.8± 0.44	7.83± 0.61^{***a,b}	9.33± 0.98^{***a,b,c}
total cholesterol (mg /dl)	162.5± 16.16	166.3± 15.7	235± 16.5^{***a,b}	264.8±15.56^{***a,b,c}
TG (mg /dl)	131.3± 8.06	136.5± 10.4	175.83± 9^{***a,b}	189.6± 14.5^{***a,b,c}
LDL (mg /dl)	85.75± 13.97	88.58± 11.63	140.83± 11.04^{***a,b}	169.16± 13.79^{***a,b,c}
HDL (mg /dl)	60.25± 3.1	57.25± 3.5	34.25± 4.65^{***a,b}	28.58± 3.5^{***a,b,c}

N = 12 rats in each group& data represented as mean and standard deviation ($\overline{X}$ ± SD**).**
(^{a})= significant versus group IA. (^{b})= significant versus with group IB, (^{c}) = significant versus group IIA. * = p<0.05 ** = p< 0.01 *** = p< 0.001

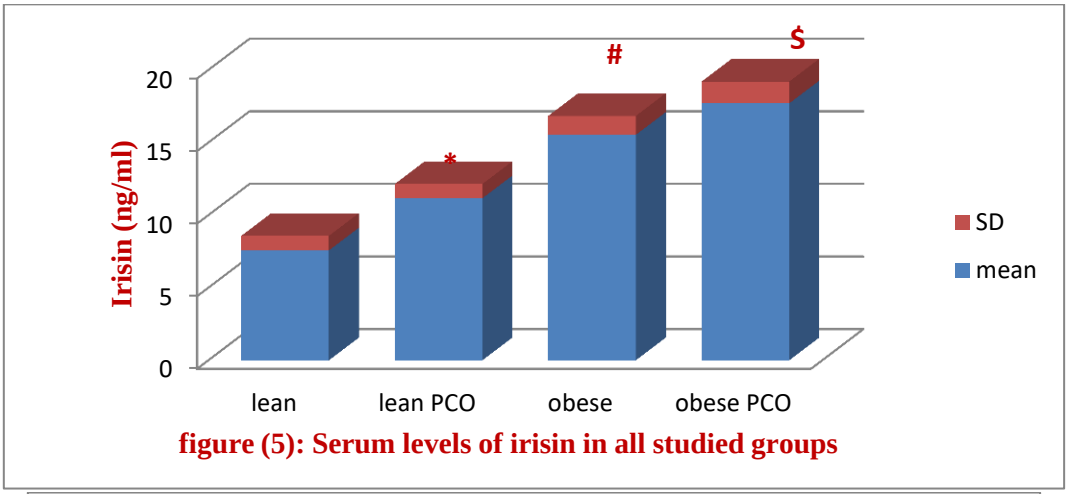

figure (5): Serum levels of irisin in all studied groups

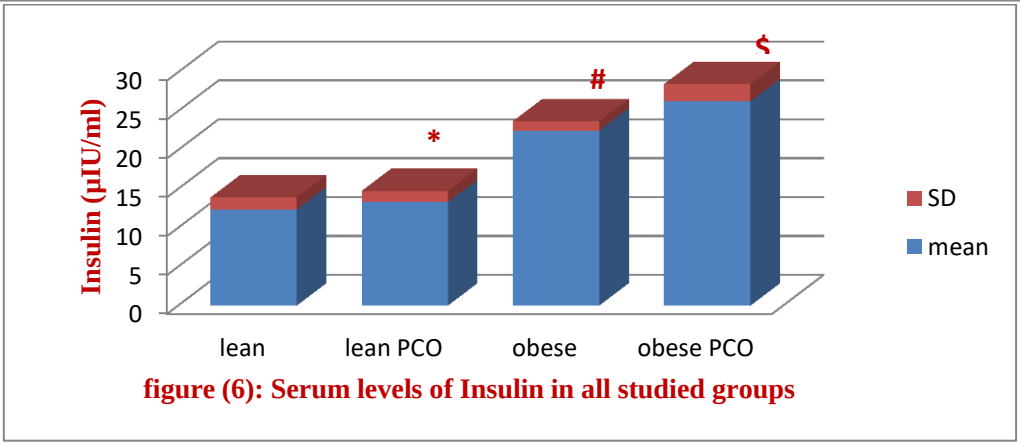

figure (6): Serum levels of Insulin in all studied groups

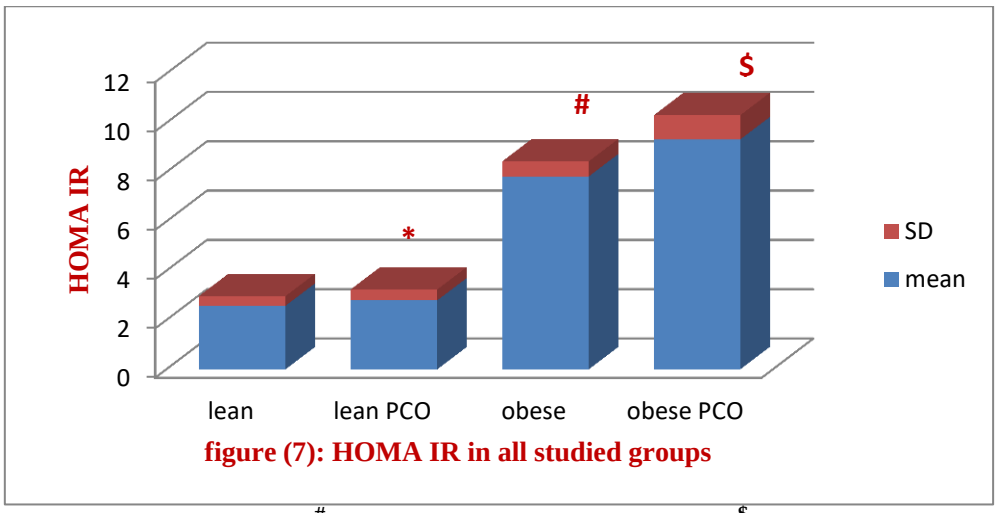

figure (7): HOMA IR in all studied groups

*** = group IB VS group IA. # = group IIA VS group IA. $ =group IIB VS group IB.**

DISCUSSION

Irisin is a myokine/adipokine with potential role in obesity and diabetes [25]. There are contradictory results about the irisin concentrations in obesity and its relation to both glucose and insulin levels and to insulin resistance [2, 3, 26].

PCOS is a health problem that affects women of childbearing period and is a common and treatable cause of infertility [27]. PCOS women suffer from disturbance in hormonal and metabolic parameters that may affect their health [28, 29].

Controversial studies were done about the levels of this myokine in PCOS and its relation to hormonal and metabolic changes of this syndrome [9,10,30]

This study was carried out to evaluate serum irisin level in letrozole-induced PCOS in both lean and obese female rats, and to find the link between serum irisin level and some metabolic and hormonal changes associated with PCOS.

In the present study, the signs of PCOS induced by letrozole in lean and obese rats was proved by the significant hyperandrogenism (higher serum testosterone level) accompanied by significant reduction in both estradiol and progesterone levels in comparison to control groups, in addition to persistent estrus and histopathological features of cystogenesis[12] .

These signs occurred because letrozole blocked cytochrome P450 aromatase which is responsible for aromatization of testosterone to estradiol [31]. Anovulation was expected because there was a decrease in serum progesterone concentrations [32], increase in the number of atretic and cystic follicles due to disturbed folliculogenesis, moreover a lack of corpus luteam and persistent estrus indicated anovulation [33].

Regarding serum irisin level, there was significant elevation in its level in both lean and obese PCOS groups in comparison with control groups. In addition, obese PCOS group had significant higher levels when compared to lean PCOS group.

This finding is consistent with Cai et al. [34] who showed that circulating irisin level was higher in PCOS patients than in overall healthy controls, and it was also elevated in PCOS patients with higher BMI than those with lower BMI. This suggested that body weight status might be a modulator of circulating irisin alterations in PCOS patients.

In addition, Bostanci et al. [35] found that serum irisin level in PCOS patients was significantly elevated when compared to control group .Li et al. [36] observed a marked increase in circulating irisin levels in obese PCOS women with high free androgen index, in parallel to an evident increase in insulin resistance and hyperandrogenemia.

In the contrary, another study showed that plasma irisin level in PCOS patients did not differ from controls and they found no relation between irisin and other metabolic parameters [10].

Also, Ali et al. [37] reported that serum irisin levels were not significantly differed from their corresponding controls, in both

obese and non-obese PCOS patients .Whereas, serum irisin levels were elevated significantly in both obese (patients and control) as compared to non-obese (patients and controls),respectively.

Another study has been found that circulating irisin was significantly lower in PCOS patients [38].

In the present study, serum irisin level showed significant positive correlation with BMI.In agreement with this finding, certain studies have reported a positive correlation between serum irisin level and BMI[39, 40], on the other hand Moreno-Navarrete et al. [2] recorded a negative correlation between serum irisin level and BMI. Kurdiova et al. [41] found no correlations between irisin and BMI.

This study showed significant increase in serum LH and testosterone levels in both lean and obese PCOS groups in comparison with control groups and no significant change in serum FSH. While, there were significant decrease in serum estradiol and progesterone levels in both lean and obese PCOS groups in comparison with control groups.

In addition, this study also showed that the increased level of serum irisin in rats with PCOS showed significant positive correlation with their testosterone levels. This means that the significant hyperandrogenemia observed in PCOS could be considered as a cause responsible for the increased of irisin level.

This may be explained as the increase in lean mass due to higher level of androgen in PCOS [42] could contribute to the secretion of circulating irisin which is secreted from muscle [1].

In addition, Acet et al. [43] found that serum irisin was positively correlated with serum level of total testosterone in PCOS patients but was negatively correlated with HOMA-IR in the overall patient population.

This study showed that serum insulin, glucose levels and HOMA- IR did not show significant change between lean and lean PCOS groups. While there was significant increase in serum insulin, glucose levels and HOMA-IR in both obese and obese PCOS groups in comparison with lean and lean PCOS groups respectively. Moreover, significant elevation in serum insulin level and HOMA- IR in obese PCOS group compared to obese group was reported.

In agreement with these results, Shi et al. [44] reported a rate of abnormal glucose tolerance in PCOS patients. Also, PCOS patients had significantly higher HOMA-IR and fasting blood glucose level compared with those of control women [45].

This can be explained as in case of obesity hyperinsulinemia and IR are accompanied with increased ovarian androgen production [46]. Other studies suggest that testosterone may worsen insulin resistance [2, 47].

This study found significant negative correlation between serum irisin level, serum insulin level and HOMA- IR in obese, lean and obese PCOS groups.

The finding in our result is consistent with Acet et al [43] who found that serum irisin level was negatively correlated with HOMA-IR in both control and PCOS patients. Furthermore Yan et al. [48] found that in obese adults, there was negative correlation between circulating irisin level and fasting insulin. In addition, a significant negative correlation between serum irisin level and each of fasting glucose level and HOMA-IR was reported in a study carried on healthy children [49].

These data can be approved by the findings of Çath et al. [50] Who found that higher level of irisin may indicate a state of irisin resistance in tissue similar to that of insulin and leptin resistance, or may be interpretated as a compensatory increase in serum irisin level in a trial to increase insulin sensitivity in obese subjects with insulin resistance.

On the other hand, Park et al. [39] reported that, in adults there was positive correlation between serum irisin level and each of fasting glucose level and HOMA-IR. Moreover, in both PCOS and normal women, circulating irisin level was positively correlated with BW, BMI, HOMA- IR [9].While, Bluher et al. [51] found no relation between serum irisin level and other metabolic parameters associated with obesity (e.g., glucose and insulin) in obese children.

There is a new prove that exogenous administration of recombinant irisin in animal models or in vitro irisin treatment in cell

culture systems is associated with enhanced glycemic control and improved insulin resistance [52, 53].

This study showed that total serum cholesterol, TG, LDL and HDL levels did not show significant change between lean and lean PCOS group. While there was significant increase in total serum cholesterol, TG, LDL and decrease in HDL levels in both obese and obese PCOS groups in comparison with lean and lean PCOS groups respectively. Furthermore, there was significant increase in total cholesterol, TG, LDL and decrease in HDL levels in obese PCOS group when compared to obese group.

In addition, there was significant negative correlation between serum irisin level and total serum cholesterol, TG and LDL levels. On the other hand, there was significant positive correlation between serum irisin level and serum HDL levels in obese and both lean & obese PCOS groups.

These results are in agreement with Tasali et al [54] who found that PCOS women have a high risk of disturbed lipid metabolism disorder.

Conflicting results have been reported between serum irisin level and lipid profile. A positive correlation between serum irisin level and TG, TC, and LDL has been recorded by Park et al. [39] and on the other hand Moreno-Navarrete et al. [2] and Yan et al. [48] found that there was no correlation between irisin and lipid parameters.

Reinehr et al. [55] observed that serum irisin level was positively correlated with LDL-C and TG levels, and negatively correlated with HDL-C in childhood.

While Al-Daghri et al. [49] reported that serum irisin level was positively correlated with HDL level in normal-weight children. Other studies showed that serum irisin level was negatively correlated with HDL level [56, 57]

Irisin may perform its effects on lipid synthesis, particularly HDL-C, but low HDL-C level may produce a direct feedback effect on myocytes, enhancing the release of irisin to restore the altered metabolism [57, 58].

Discrepencies in these results and other may be related to difference in species, irisin kits, study design, and duration of the experiment.

In conclusion: High serum irisin level in PCOS and obese groups were negatively correlated with insulin resistance and hyperlipidemia, while positively correlated with BMI and hyperandrogenemia. Irisin may have a role in the development of polycystic ovary syndrome. Moreover, the finding of high serum irisin level in PCOS may provide a novel biomarker for detection of this syndrome.

RECOMMENDATION

Further studies are needed to clarify the exact role of exogenous administration of irisin in hormonal changes in infertility.

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