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Descriptive Consideration of Serum Irisin Levels Various Factors: Obesity, Type 2 Diabetes Mellitus, Pre-Diabetic Status, Gender, and Athletics

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Abstract

Aim: Recently obesity, has become the main risk for a number of conditions and metabolic syndrome including type 2 diabetes (T2DM). The aims of this study were to assess whether irisin can bring out the missing circle between increasing physical activity and weight reduction.

Methods: 108 subjects were chosen to matched with 76 diabetic patients in age and BMI, both groups were divided into subgroups according to their BMI.22 healthy athlete men were also involved in the study.

Results: The mean (± SD) value of serum irisin concentrations of obese healthy individuals was the highest concentration (p=0.015) in comparison with normal and overweight healthy individuals. The more recent observation which describe irisin as adipokine factor (willing myokine source), irisin levels were examine pursuant to gender, obese women group have had the highest level of serum irisin than other women studied groups (p=0.001), while in healthy men serum irisin did not differ significantly. Carful examination were searched for irisin as a myokine after adjusting age and BMI for gender depending on that women had significantly lower serum irisin (p=0.006) inspite of signficantly higher fat precentage (p=0.001) when compared with men. signficantly lower serum irisin concentration in healthy men with normal physical activity (p=0.01) than healthy athlete men pointed that irisin as myokine potentionally. Diabetic patients showed significant lower serum irisin level than healthy subjects.

Conclusion: Serum irisin levels findings may reflect its protective role against developing insulin resistance.

Keywords: Irisin; Exercise; Physical activity; Obesity; Type 2 Diabetic; Obesity treatment; Weight reduction

Introduction

The Rate of obesity is a peril evolution worldwide [1], lifestyle environmental factors and low physical activities may play a dominant role in obesity development. Central obesity, the main risk factor for a number of conditions and metabolic syndrome including type 2 diabetes mellitus (T2DM) [2,3]. Insulin resistance plays an important role in the pathogenesis of T2DM. Skeletal muscle accounts for the majority of glucose uptake in response to insulin and it is an important site of insulin resistance [4]. Chronic exercise training induces adaptive structural and metabolic changes in skeletal muscle including a change in the type of muscle fiber, mitochondrial biogenesis, and angiogenesis [2]. Forceful progress is developing the molecular regulatory circuitry in the exercise-induced changes in muscle structure and function, the expression of a gene transcriptional co-regulator called peroxisome proliferator-activated receptor ɣ (PPARɣ), co-activator-1α (PGC-1α) is induced in muscle in response to exercise in rodents and humans [5,6]. PGC-1α over expression in muscle, cause changes in whole body fat tissue depots. Profiling of muscle genes activated by PGC-1α identified a factor called fibronectin type III domain containing 5 (FNDC5) with predicted structural features of type I membrane protein that could be proteolytically cleaved to release a smaller protein into the bloodstream, a secreted protein product of FNDC5, named irisin was found [7]. For most people attempting to lose weight, chronic/regular physical activity is an important causal of successful long-term weight loss [8]. Daily energy expenditure guided for more rapidly obesity disappears. The link between physical activity to energy metabolic homeostasis is perhaps irisin, irisin is the fasten factor between myocyte-adipocytes connection. The circulating factor irisin, cleaved from FNDC5, activates thermogenic programs in white adipose tissue (browning) including mitochondrial biogenesis and expression of uncoupling protein (UCP1) [7]. The browning of WAT is associated with augmented mitochondrial density and oxygen consumption producing heat leading to favorable effect on [9].

Irisin discovery may raise hopes regarding the hypothesis that it may provide a new treatment for obesity and diabetes. That peptide hormone may hold additional benefits for a wide range of pathological conditions since this hormone may be therapeutically and clinically beneficial [10]. The aims of this study were 1) to assess whether irisin is the link circle between increasing physical activity and weight reduction, increasing insulin sensitivity and improvement of DM, and prevention of pre-diabetic status, 2) to show the included mechanism and 3) to evaluate its effectiveness as adipokine to myokine.

Methods

Study designed: This human study model was carried out at Biochemistry Department, College of Medicine, University of Baghdad , Al-Yarmok Teaching Hospital, Obesity Center at Al-Kindy Medical College, and Athletic College, University of Baghdad, Iraq, during the period from January 2013 to July 2013.

Investigations included serum measurements of irisin, insulin and leptin using ELISA technique. HbA1c, serum fasting glucose and lipid profile parameters including total cholesterol, triglyceride (TG), Low-density lipoprotein-cholesterol (LDL-C) and high-density lipoprotein-cholesterol (HDL-C) were also measured using spectrophotometric methods. All these measurments were done at the Laboratories of Biochemistery Department, Medicine of College, University of Baghdad and at the Teaching Laboratories, /Baghdad Teaching Hospital, Medical City, Baghdad, Iraq. All the enrolled subjects were in overnight fasting state.

Subjects

Patients: one hundred T2DM patients were encountered, but only seventy six were included in the current study to be age and body mass index (BMI) matched with healthy individuals group. Thirty-four of these patients were overweight (15 women and 19 men), their BMI were > 25 -29.9 kg/m², with age range of 35-67 year, forty-two were obese (21 women and 21 men), their BMI > 30 kg/m², and their age range was 38-62 year. The criteria for diagnosis and inclusion of these patients with type 2 DM was depended on careful history and/or the measurements of serum glucose and HbA1c [11].

Healthy subjects: Two-hundred healthy subjects were encountered, but only 108 of them were enrolled to matched the 76 diabetic patients in their age and BMI, This group was subdivided into groups according to their BMI: [12]. forty-one normal weight healthy subjects group (21 women and 20 men), their age range was (38- 59 year) and their BMI was > 18 kg/m²-≤ 25 kg/m, 27 over weight healthy subjects group (12 women and 15 men), their age range was between 39 to 60 year and 40 obese healthy subjects group (20 women and 20 men), their age range was between 39 to 61 year. All these subjects were on normal activity range (20- 60 min/day).

Healthy athlete subjects: This group included 30 men, with only 22 of them to be age-matched with healthy non-athlete subjects that were included in this study. These athletes were of different kinds of sport.

Exclusion criteria included those who have type I DM, thyroid disorders, Cushings syndrome, renal diseases, ischemic heart disease and pregnant women. The exclusion was achieved by accurate clinical history and laboratory analysis under the supervision of consultant physician in internal medicine.

Blood Sampling

Five-seven millliter (ml) of blood was collected from the peripheral vein of each enrolled subject after overnight fasting state, 2.5 ml was transferred into anti-coagulant tube for HbA1c measurement, [13] and the remaining blood was put into vancover tube, it was allowed to clot and centrifuged for 15 min at 3000 rpm to obtain clear serum which was stored at -20°C till the time of estimation of serum irisin [14], leptin [15], insulin [16], glucose, and lipid profile parameters [17].

Kits for serum irisin, cat. No. EK-067-52 and human insulin, cat. No. EK-035-06 measurements were provided by Phoenix Pharmaceuticals. INC, USA. Human Leptin (LEP) ELISA kit, catalog No.CSB-E04649h was supplied by CUSABIO BIOTECH CO., LTD. CHINA. Kits for HbA1c, serum glucose and the lipid parameters were obtained from Human Company, Germany.

Anthropometric measurments including BMI (kg/m²) was calculated by dividing weight (kg) by height squared (m²), fatpercentage was also calculated by the following equation [18]:

Adult body fat percentage = $(1.20 \times BMI) + (0.23 \times Age) - (10.8 \times Sex) - 5.4$

Male = 1, Female = 0.

Statistical Analysis

Statistical package for social science (SPSS) version 19.0 for Windows program on the computer was used to compare varibles ,which were expressed as mean ± SD. Student' s t- test was employed to compare the means of normally distributed studied variables between two groups, while ANOVA (f-test) was used to detemine the difference among more than two groups. Pearson's correlation was used to determine if the studied parameters were related to change in other studied parameters in the same group. Selected percentiles were depened to determine the cut-off value which represent the critical point in developing insulin resistance.

Results

The results of this study revealed the effect of obesity on serum levels of recent exercise peptide or hormone, the irisin and related metabolic factors in healthy subjects. The healthy subjects were selected to be classified into three age matched groups according to their BMI, normal weight (22.74 ± 1.63 kg/m²), overweight (27.34 ± 1.21 kg/m²) and obese (35.54 ± 5.27 kg/m²) groups, with highly significant differences in their BMI (for all, P=0.001), the mean of the fat percentage was also differ significantly among these healthy groups with a value of (27.03 ± 5.38%) for normal weight, (32.05 ± 5.8%) for overweight and (42.3 ± 8.88%) for obese (for all, P=0.001).

Circulating irisin level was significantly higher in healthy obese subjects (49.58 ± 7.55 ng/ml) compared with healthy normal weight subjects (42.8 ± 14.3 ng/ml, P=0.009) and healthy overweight subjects (45.97 ± 6.02 ng/ml, P=0.03). Overweight healthy subjects also had significantly high serum irisin than normal weight healthy subjects (P=0.001). The other studied metabolic hormones, serum leptin level showed a significant increase in healthy obese subjects against healthy normal weight subjects (28.9 ± 20.9 ng/ml vs. 23.3 ± 15.1 ng/ml), respectively; p=0.0001), while serum insulin and serum fasting glucose showed no significant differences among studied groups as shown in Table 1. The result also clarified that there was no significant differences

Studied parameter	Normal weight healthy subjects (n=41)	Overweight healthy subjects (n=27)	Obese healthy subjects (n=40)
Fasting serum NS glucose (mg/dl)	89.45 ± 17.04	87.81 ± 9.82	89.95 ± 13.55
Serum irisin (ng/ml)	42.76 ± 14.28	45.97 ± 6.03▲ ▲	49.58 ± 7.55▲
Insulin (µIU/ml) NS	20.9 ± 8.1 (n=23)		22.45 ± 7.1 (n=30)
Leptin (ng/ml)	23.3 ± 15.1 (n=23)		28.9 ± 20.9● (n=30)

ANOVA and t-test revealed, NS no significant differences in fasting glucose and insulin among the groups, ▲; t-test showed significant difference between the obese and normal weight groups (P=0.001), ▲ ▲; t-test revealed significant difference between overweight and normal weight groups (P=0.009), ●; t-test showed significant difference between obese and normal weight groups (P=0.001).

Table 1: Serum Irisin and related metabolic factors in healthy subjects (insulin and leptin), mean distribution values.

in studied lipid profile parameters among and between studied healthy groups, all had p > 0.05 (data not show).

Serum fasting irisin was correlated significantly positively with (a) BMI (r=0.32, P=0.001) (b) serum HDL-Cholesterol (r=0.83, P=0.0001) and significantly negatively (r=0.42, P=0.003) with level of serum insulin in the whole group of healthy subjects (Figure 1). Serum fasting irisin changes in healthy subjects did not depend on the changes in serum leptin, fat percentage and other lipid types, P>0.05, however serum irisin was negatively correlated with age but did not reach the significant level.

Irisin as myokine factor was masked to reflect more accurate descriptive results about serum irisin level in the healthy subjects as adipokine. Therefore healthy subjects were subdivided according to their gender and their BMI into age-matched healthy women (normal weight, overweight and obese) and healthy men (normal weight, overweight and obese). Successful selection of healthy women according to their BMI was reflected by highly significant differences among and between studied groups (P=0.0001) as well as Fat percentage (P=0.0001). Serum irisin of obese healthy women was highly significantly increased (48.78 ± 7.41 ng/ml, p=0.0001) in comparison to that of normal weight healthy women (36.79 ± 11.69 ng/ml), but did not had significant difference against overweight healthy women (45.23 ± 5.03 ng/ml). The other metabolic hormones also showed highly significant difference that obese healthy women had higher serum insulin and leptin level than normal weight healthy women (23.75 ± 2.20 µIU/ml vs. 19.84 ± 0.99 µIU/ml, P=0.001 and 30.69 ± 5.3 ng/ml vs. 15.48 ± 4.27 ng/ml, P=0.0001) consequently, (Table 2).

Serum irisin level in healthy obese women with fat percentage >32 (46.77 ± 8.22) was comparable with that of healthy normal weight women with fat percentage ≤32 (30.48 ± 5.36). With respect to men groups, the mean of serum irisin levels as well as that of insulin and leptin did not differ significantly among groups of different BMI and fat percentage (data not show). In order to evaluate irisin peptide as a myokine factor, the whole healthy subjects group was also subdivided according to (the study's design)their gender into two groups, women and men, there were no significant differences in age (p=0.70) and BMI (P=0.877). Despite that the mean of fat percentage value of women (39.39 ± 8.55%, P=0.001) was significantly higher than that of men (28.72 ± 7.59%), their serum irisin levels was significantly decreased (43.22 ± 10.11 ng/ml vs. 48.86 ± 10.61 ng/ml, respectively; p=0.006), while other related metabolic factors like serum insulin and leptin showed non-significant differences between men and women as shown in Table 3.

The role of muscle in synthesis and secretion of irisin was emphasized by the comparison of its mean value between athlete males and age-, BMI non-significant difference in their age, BM & fat percentage, but T2DM patients had lower circulating Irisin (42.9 ± 11.8 ng/ml P=0.001), higher serum insulin (20.10 ± 6.08 µIu/L), P=0.04) with higher HOMA- IR(7.1 ± 2.5, P=0.001) and serum leptin (42.1 ± 18.4 ng/ml, P=0.001) against studied healthy subjects (48.13 ± 7.15 ng/ml), (16.84 ± 7.65 µIu/L), (3.7 ± 0.93) and (29.3 ± 5.5 ng/ml), consequently.

Lipid abnormalities resulted from diabetic complication and consequent for insulin resistance in T2DM patients, they had significant increase of TG (200 34.5 mmol/l, P=0.001 and significant decreased of HLDL-Cholesterol (40.01 10.40 mmol/l, P=0.006) against the healthy subjects group (171.3 29.4 mmol/l, 45.58 9.73 mmol/l, respectively). The other lipid parameters (total cholesterol and LDL-cholesterol) did not change significantly between these two groups.

As well as the healthy subjects who were selected to be classified into groups according to their obesity, the same way of classification was used for the diabetic patients. The diabetic patient groups (over weight and obese) were age-, BMI- and fat percentage -matched for

Studied Parameter	Normal weight healthy women	Overweight healthy women	Obese healthy women
Irisin (ng/ml)	36.79 ± 11.64 (n=21)	45.23 ± 5.03▲▲ (n=12)	48.78 ± 7.41▲, NS (n=20)
Insulin (µIU/ml)	23.75 ± 19.84 (n=10)		19.84 ± 0.99▲ (n=14)
Leptin (ng/ml)	15.48 ± 4.27 (n=10)		30.69 ± 5.3● (n=14)

ANOVA and t-test revealed ▲ significant difference between the obese and normal weight women groups (P=0.0001), ▲▲ significant difference between overweight and normal weight women groups (P=0.01), ● significant difference between obese and normal weight women groups (P=0.001), NS: No significant difference between obese and overweight women groups.

Table 2: Serum Irisin level in healthy studied women groups depending on their BMI (± SD).

Studied Parameter	Healthy men (n=55)	Healthy women (n=53)	P-value
Fat percentage	28.72 ± 7.59	39.39 ± 8.55	0.001
Irisin (ng/ml)	48.86 ± 10.61	43.22 ± 10.11	0.006
Insulun (µIu/L)	19.66 ± 5.43	20.93 ± 6.25	NS
Leptin (ng/ml)	21.82 ± 19.15	31.49 ± 19.61	NS

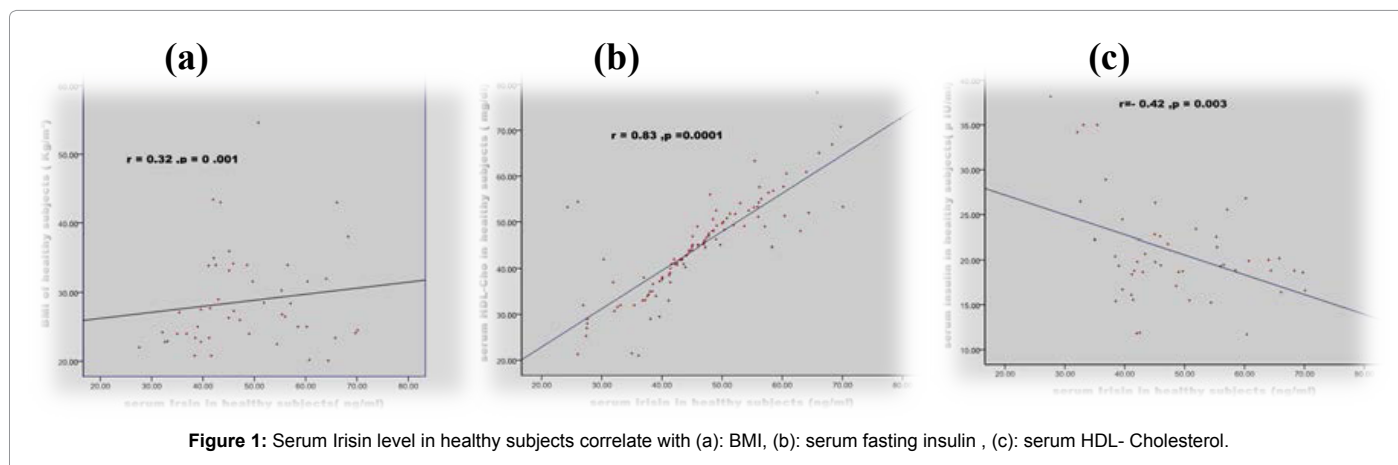**Table 3:** Serum Irisin difference depending on gender.

Figure 1: Serum Irisin level in healthy subjects correlate with (a): BMI, (b): serum fasting insulin , (c): serum HDL- Cholesterol.

their overweight and obese healthy control groups. Lower serum irisin level was associated with overweight type 2 diabetic patients, as shown in Table 6, with highly significant difference (37.06 ± 10.89 ng/ml) when compared with overweight healthy subjects (45.97 ± 6.03 ng/ml), obese healthy subjects (49.58 ± 7.55 ng/ml) and obese type 2 diabetic patients (47.56 ± 10.49 ng/ml, for all; p=0.001). While the serum irisin level of obese diabetic patients was lower than that of obese healthy subjects, but did not reach the significant level even when they had significant increase in serum insulin concentration (18.0 ± 6.08 μIU/ml) and serum leptin (42.03 ± 19.54 ng/ml) against obese healthy subjects (19.69 ± 2.78 ng/ml, 25.34 ± 17.14 ng/ml, respectively) (for both, P=0.001). Table 6 revealed among other findings that overweight DM patients with long diabetes duration had poor glucose control as their HbA1c % which was highly significantly increased (8.32 ± 2.09%, p=0.001) when compared with obese DM subjects who had lower diabetes duration and well controlled (6.84 ± 1.11%).

In the whole group of diabetic patients, there was significant negative correlation between serum fasting irisin and HbA1C (a) with (r= -0.29, P=0.01). Moreover, Serum fasting irisin in T2DM patients was correlated significantly positively with (b) serum insulin, (c) serum leptin (r=0.51, P=0.0001) and (r=0.33, P=0.03; subsequently).

Depending on the selected statistical 10th percentile value in obese healthy subjects as shown in Table 7, the study found that the proposed cut-off of serum irisin levels for identification of those asymptomatic subjects with high risk for developing type 2 diabetes mellitus was 41.4 ng/ml. The study also revealed the non-significant difference in mean serum irisin levels between the 10th percentile obese healthy subjects (40.78 ± 0.68 ng/ml) and those who had established type2 DM (42.86 ± 11.8 ng/ml) along with highly significant difference between diabetic patients and obese healthy control subjects with percentile between 10th-90th (48.97 ± 5.29 ng/ml, P=0.002). The association of new peptide irisin with other metabolic hormones (insulin & leptin) which are involved in pathogenesis of type 2 DM was illustrated in Table 7. The serum insulin and leptin levels did not differ significantly between the obese healthy subjects with irisin percentile value below 10th and diabetic patients, but there was a significant increase in both hormones in diabetics than in obese healthy subjects 10th-90th irisin value (insulin, P=0.001 & leptin, P=0.007). Also serum insulin was significantly increased in obese healthy controls who have high risk for type 2 DM (serum Irisin value ≤ 41.4 ng/ml) compared with obese healthy control subjects (serum irisin value 41.4 ng/ml). With regard to HOMA-IR, diabetic patients group had significantly higher level (6.8 ± 3.32) than both obese healthy subjects; those with serum irisin ≤ the 10th percentile (4.5 ± 2.33, P=0.03) and between the 10th-90th percentile (2.33 ± 1.1, P=0.004).

Discussion

The result in Table 1 revealed a positive increment in circulating irisin with total body adiposity in healthy subjects, which tend to be higher in obese than in over weight and normal weight healthy subjects documented by a positive significant correlation between serum irisin and BMI as shown in Figure 1a. These results are similar to those obtained by Ivanov et al. [19], Stengel et al. [20], Wen et al. [21] which showed that irisin was correlated positively with BMI and other most known markers of insulin resistance in healthy non-diabetic subjects, this could indicate that increased circulating irisin is an adaptive response in metabolism associated with obesity [19].

The above finding of the present study confirmed those found by Stengel et al. [20]. Huh et al. [22] whose results indicated that

Studied Parameter	Healthy Men (n=40)	Healthy Athlete Men (n=22)	P-value
Age (year)	45.03 ± 5.64	43.82 ± 12.20	NS
BMI (K/m²)	30.24 ± 4.54	29.33 ± 4.92	NS
Fat percentage	30.44 ± 5.82	28.39 ± 6.95	NS
Irisin (ng/ml)	49.83 ± 9.55	60.37 ± 12.31	0.001

Table 4: Myokine origin of serum Irisin.

Studied Parameter	Overweight and obese healthy subjects (n=67)	Overweight and obese T2DM patients (n=76)	P-value
Age (year)	45.88 ± 5.46	47.79 ± 7.51	NS
BMI (kg/m²)	37.32 ± 5.78	31.67 ± 4.72	NS
Fat percentage	38.19 ± 9.24	37.89 ± 8.48	NS
Irisin (ng/ml)	48.13 ± 7.15	42.90 ± 11.8	0.001
Insulin (μIu/L)	16.84 ± 7.65 (n=30)	20.10 ± 6.08 (n=59)	0.04
Leptin (ng/ml)	29.30 ± 5.5 (n=30)	42.1 ± 18.4 (n=59)	0.001
HOMA-IR	3.7 ± 0.93 (n=30)	7.1 ± 2.5 (n=59)	0.001

Table 5: Base line characteristics and biochemical profiles of healthy subjects & T2DM patients.

Studied Parameter	Overweight healthy subjects (n=27)	Overweight T2DM patients (n=34)	Obese healthy subjects (n4=40)	Obese T2DM patients (n=42)
Irisin (ng/ml)	45.97 ± 6.03▲	37.06 ± 10.89 NS	49.58 ± 7.55 NS	47.56 ± 10.49 ▲
HbA1c %		8.32 ± 2.09		6.84 ± 1.11▲
Duration of DM (month)		87.21 ± 68.10		27.21 ± 24.82▲

ANOVA and t-test revealed ▲ significant differences between obese T2DM and overweight T2DM patients, between overweight healthy subjects and overweight T2DM patients (P=0.001). NS: No significant differences between each of the obese healthy and overweight healthy subjects.

Table 6: Determination of the probability distribution and information about significant differences of serum irisin, HbA1c and duration of DM in diabetic patients and healthy subjects.

Studied Parameter	Obese healthy subjects with < 10th irisin percentile value (GI)(n=9)	Obese healthy subjects with irisin percentile value between 10th -90th (GII) (n=31)	Established T2DM patients (n=76) (GIII)
Irisin (ng/ml)	40.78 ± 0.68▲	48.97 ± 5.20 NS	42.86 ± 11.8
Insulin (μIu/L)	17.80 ± 5.77 NS	15.93 ± 4.74 ▲ ▲	18.0 ± 6.08
Leptin (ng/ml)	30.73 ± 18.54 NS	24.95 ± 15.55●	42.03 ± 19.54

ANOVA and t-test showed ▲significant differences between GI and each of GII and GIII as well as between GII and GIII (P=0.002), ▲ ▲significant difference between GII and GIII (P=0.001), ● significant difference between GII and GIII (P=0.007). NS: No significant differences between GI and each of GII and GIII as well as between GII and GIII.

Table 7: Cut-off risk level of serum irisin for type 2 DM.

morbidly healthy obese individual had higher circulating levels of irisin than normal weight, their conclusion depend on the fact that decrease in irisin levels after weight loss was explained by the *decrease in muscle mass* in normal healthy subjects suggesting that the positive association of irisin with BMI is at least in part explained by muscle mass. In the authors opinion, this could indicate that increased in circulating irisin is an adaptive response needed to *compensate* for the *decreasing insulin sensitivity* and disturbances in metabolize associated obesity. A study performed by Huh et al. [22] strongly supports the above findings, the authors found higher circulating irisin level in obese patients compared with normal weight, which resulted in a correlation between irisin, body weight and fat mass among other variables, the interpretation produced by these authors suggested that circulating irisin is affected under conditions of altered BMI, with the highest levels in obese patients. More recently Roca-Rivada et al. [23], proposed that the muscle/adipose irisin secretion ratio may depended on the physiological status, that with exercise training muscle tissue would strongly affect the FNDC5 circulating levels, whereas, in a typical BMI cases such as obesity, adipose tissue would actively elevate FNCD5/ irisin [24]. One major challenge for future advances in the understanding of *irisin a specific, high –affinity receptor* is supported by low–non molar activity of the protein [25] and the knowledge of its identity would likely unravel new possible target cells/tissues for irisin action. Moreover, the notion of a potential *irisin –resistance* in obesity, Type 2 DM and other various disease state could be directly tested if the receptor system is known [26].

In contrast to the findings presented by the current study and above mentioned studies, Liu et al. [27] reported that circulating irisin correlated negatively with BMI, waist-hip ratio and fat mass in men. The result presented in Table 3 of the present study may add a new discrepancy by finding no significant differences in serum irisin level when fat percentage was depended on as the base of division, fat percentage is a well indicator of total adiposity than BMI as it included age and sex in addition to BMI in its measurement [11,12]. However, in the report of Liu et al. [27], there was no measurement of body composition to directly test the influence of differences in muscle mass [22]. These discrepancies could reflect on gender-differences, and also suggest a dissociation between FNDC5 expression and circulating irisin under some condition [7]. The data presented in Table 2 may answer the above suggestion related to gender-irisin association which revealed that women serum irisin level was affected by the changes in obesity marker, the BMI values with higher serum irisin was higher in obese and overweight healthy women than in normal weight healthy ones. In men this changes do disappear. These results could be explained by accompanied significant increase of serum leptin and insulin levels in obese healthy women than in normal weight healthy women (Table 2) and the role of irisin elevation to compensate for hyper-insulinemia and the expected insulin resistance.

The results of serum irisin levels in studied healthy subjects and T2DM patients groups pointed that its level may be altered during the course of the disease like obesity and associated metabolic disorders. Table 5 showed through overt results that studied T2DM patients had lower irisin level than healthy subjects. There are many suggestions made by very few studies recently published in 2013-2014 that interpreted the low serum irisin level in their diabetic patients. A study done by Choi et al. [28] reported that disrupted irisin signaling in adipose tissue may be associated with the development of T2DM, their data suggest that decreased serum irisin levels may be associated with the development of insulin resistance and T2DM [29,30], suggesting specific control patterns of Fndc5/irisin in muscle, myotubes, adipose

tissue and blood in obesity, pre-diabetes and type 2 diabetes. This pattern points to complex and tissue-specific mechanisms, resulting into timely regulation of local and circulating irisin level, which might be important in determining metabolic health and disease conditions. In addition, the findings of this study observed that muscle expression of FNDC5-irisin, was gradually up regulated with increasing obesity and glucose intolerance in non-diabetic individuals, thus compromising the proposed positive role of irisin in metabolic disease. In humans, adipose tissue FNDC5 expression may represent only a fraction of that expressed in muscle, but it is adipose tissue not skeletal muscle expression that is associated with the levels of circulating irisin [31]. Similarly, Pekkala et al. [30] found that expression of FNDC5 in subcutaneous adipose tissue was nearly 100 times lower than in skeletal muscle as determined by RT-PCR technique. The authors revealed that pre-diabetic and diabetic's type 2 expressed 40-50% less FNDC5 in subcutaneous adipose tissue compared to healthy obese men, which paralleled the reduction of blood irisin in type 2 diabetic patients. A new insight of diabetes –associated poor exercise proposed by Ivanov et al. [19] and Huh et al. [22] revealed that exercise is involved in the link between irisin and beta-trophin with subsequent beta–cell regeneration and decrease in insulin resistances.

Earlier studies found that the expression of PGC-1α and its activities were reduced in skeletal muscle in type 2 diabetics [32,33]. These findings suggested that circulating irisin may be lower in type 2 DM patients due to lower PGC-1α activity in skeletal muscles [34].

Table 6 showed in details, the differences in serum irisin level among studied groups, overweight diabetic patients had lower serum irisin level than obese diabetic patients with higher duration and lower glycemic control. The study may reflect on the fact of the significant contribution of long term uncontrolled DM on serum irisin level irrespective of BMI, these results are in line with the results of Liu et al. [27], who pointed that long-term diabetes is associated with significant reduction in levels of irisin. Another study speculated that serum irisin level could be affected by BMI or insulin resistance in advanced stage of diabetes [22]. Of interest in this context is that patients with early–onset T2DM display abnormalities in the exercise–dependent pathway that regulates the expression of PGC-1 [29]. Thus lower circulating irisin in individuals, who develop T2DM could play an important role for the development of the disease [22]. The significant decreased of serum irisin level in overweight diabetics than in obese diabetics of so-called exercise resistance was observed in these recent studies.

One of the most important findings of the present study that give the motivation to evaluate the cut-off value of serum irisin which was statistically derived to be 41.40 ng/ml was that serum irisin could be considered as a biochemical marker for the risk point for developing diabetes in obese healthy individuals, but this finding need to be confirmed by further future studies. Obese healthy subjects with an irisin mean value of ≤ 10th percentile value (40.78 ± 0.68 ng/ml) can be considered as pre-diabetic index in individuals whom had higher insulin and leptin levels and which may develop insulin resistance when compared with obese healthy subjects of 10th-90th of irisin value (Table 7). This observation emphasized the speculation of lower serum irisin level in T2DM patients and those with high risk. These data show that the patients are either in the diabetic state itself or they are in the metabolic condition that caused a progression toT2DM which is accompanied by decreased serum irisin concentration. Also, of plausible explanation could be the lower abundance of PGC-1α in skeletal muscle reported in condition with marked insulin resistance such as women with PCOS, T2DM, and their off-spring [35,36]. Taken

in our mind the important role of PGC-1α-FNDC5-irisin in induction of GLUT4 protein level, so their limitation in obese muscles may impair the insulin stimulated glucose transport and insulin signaling [29]. In the same Table 7, the high serum level of irisin in obese healthy subject's 10th-90th group may be explained by the fact of gradual up-regulation of this muscle peptide with increasing obesity and expected insulin resistance in non-diabetic individuals indicating the proposed positive role of irisin in metabolic disease. Increased irisin levels could therefore potentially modulate deteriorated insulin sensitivity, particularly in obese sedentary individuals.

The previous findings of the present study highly pointed that circulating irisin of healthy individuals may be of adipokine source less than myokine. To prove that well, serum irisin level was investigated in relation to muscle factors; the gender and exercise excluding other affected factors like BMI and age. Table 3 showed that irisin level in men is greater than women even fat percentage in women is greater (estrogen surge in female is responsible for the increase in essential body fat in women). It is well documented that the muscle mass (physical size) of men is greater than that of women. Men usually have twice muscle cells and more faster twitch muscles fibers than women (Gutt). In support of this view, Stengel et al. [20] and Pekkala et al. [30] reported that an increase in muscle mass was accompanied by increased PGC-1α and FNDC5 expression in muscle, increased irisin levels in blood.

These latter finding showed the gender effect irrespective of exercise, Table 4 viewed the serum irisin level by muscle mass assessment in the same gender depending on physical activity, with higher serum irisin level in chronic healthy athletics men than age, BMI, sex and fat percentage matching non – exercised healthy men. Laaksonen et al. [34] and Irving et al. [37,38] suggested that PGC-1α is strongly expressed in human skeletal muscle and can be induced by endurance training. The release of irisin from FNDC5 to the extracellular space has also been reproduced in a number of studies, [22,31] the regulation of irisin by exercise, however, has been reproduced only in some cohorts, whereas a lack of regulation was seen in others done under different physiological and/or experimental conditions [1].

Another aspect that remains to be clarified is the timing of irisin increase after exercise; since the above studies have tested irisin in different time before and after exercise the possibility exists that irisin increases for a finite period of time after exercise but its levels do not stay elevated for a prolonged period of time [36]. Another study conducted by Handschin and Spiegelman [39] and Wenz et al. [40] showed the positive beneficial effect of exercise in muscle on PGC-1α induction which in turn stimulates mitochondrial biogenesis, angiogenesis, and fiber-type switching. It also provides resistance to muscular dystrophy and the health benefits of elevated muscle expression of PGC-1α may go beyond the muscle itself. PGC-1α stimulates the secretion of factors from skeletal muscle that affect the function of other tissues via stimulating the expression of several muscle gene products including FNDC5-irisin. Irisin is induced in exercise and it activates profound changes in subcutaneous adipose tissue stimulating browning and UCP1 expression.

According to observations in Figures 1 and 2 serum irisin level was significantly positively correlated with BMI in healthy subjects group, while its change was independent in diabetics group. However, the age of healthy subject and diabetic groups show independent negative association with serum irisin levels. These finding was similar to that obtained by Huh et al [22], Park et al. [25], Liu et al. [27], Moreno-Navarrete et al. [31] regarding the healthy subjects group but not diabetics group in whom significant negative correlations were found. Moreover and in agreement with the present study, Choi et al. [28], Ogasawara et al. [40] did not observed any significant correlation between serum irisin levels and both the age and BMI in their diabetics group.

In contrast, serum irisin levels were found to be significantly negatively correlated with age in both healthy and diabetic subjects [22,41]. In addition, regarding healthy subjects group, serum irisin was also significantly negatively correlated with serum insulin which may reflect the role of PGC-1α-FNCD5-irisin in enhancing the insulin action and sensitivity against its resistance and/or maybe by increasing the GLUT4 expression pointing to its response to increased burden of metabolic dys-regulation in non-diabetic populations. The significant positive association of irisin with HDL-cholesterol in healthy non diabetic subjects may reflect a new insight on protective role of irisin against ischemic heart diseases. PGC-1α-irisin targeting of sub-sarcolemmal mitochondria may be an attempt to efficiently remove, via beta-oxidation, fatty acids as soon as they cross the sarcolemma, thus minimizing their intramuscular accumulation with consequent improvement of lipid metabolism [36].

In consistent with the results of this study, Ogasawara et al. [40], and Sanchis-Gomar et al. [41] did not find any significant correlations of total serum cholesterol, serum triglycerides and serum LDL-cholesterol levels with serum irisin. However, Wen et al. [21] revealed that serum irisin was significantly positively correlated with total serum cholesterol, serum triglycerides and serum LDL-cholesterol and fasting blood glucose in non-diabetic populations and attributed
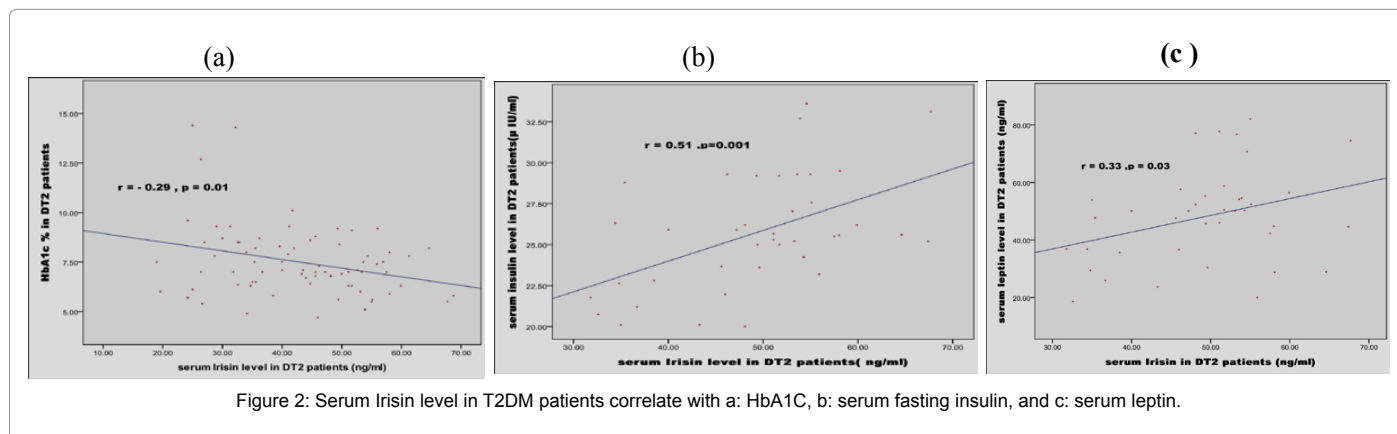

Figure 2: Serum Irisin level in T2DM patients correlate with a: HbA1C, b: serum fasting insulin, and c: serum leptin.

to its influenced by positive energy metabolism. In diabetics group, this study reported for first time the significant positive correlations between serum irisin and both of insulin and leptin which may be explained by the mechanism that obesity induced by lepteinemia leads to insulin resistance and hype-rinsulinemia accompanied by concurrent increased in irisin secretion and blood levels with probability of subsequent adipose tissue irisin signaling disturbance. The significant negative correlation of serum irisin and HbA1c that was found in this study was in accordance with that reported by Hittel et al. [42] who postulated that a relationship exists between the serum irisin levels and glucose metabolism with positive regulatory effect of irisin.

Conclusion

High serum Irisin level in healthy obese subjects and low serum Irisin level in T2DM patients may reflect its protective role against developing insulin resistance. Certain value (cut-off) of irisin levels may foretell to prediabetic status. Irisin myokine in facing irisin adipokine is powerful; that women had lower Irisin level even with higher fat percentage, in addition athletics healthy men had higher serum Irisin than the same gender with normal activity.

Acknowledgments

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