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EFFECT OF CHRONIC AEROBIC EXERCISE TRAINING ON SERUM IRISIN LEVEL IN TYPE 2 DIABETIC RATS

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ABSTRACT

Background: Exercise and diet play a key role in management of type 2 diabetes. Skeletal muscles secrete a variety of substances named myokines that are believed to mediate the beneficial effect of exercise. Irisin is a peptide secreted by skeletal muscles in response to exercise and also by adipocytes. So, it can be considered as myokine and adipokine. Irisin was reported to have an influence on glucose homeostasis and lipid metabolism.

Objective: To study the effect of chronic aerobic swimming exercise on serum irisin levels in type 2 diabetic rats, and clarify the possible association between these levels and some metabolic parameters.

Material and Methods: The present study was carried out on a total number of 40 adult male albino rats divided into four equal groups: group I (standard diet sedentary group): animals were fed normal laboratory chow diet with no exercise training; group II (standard diet exercised group): the rats were assigned to chronic swimming exercise protocol of moderate intensity (1 h /day, 5 days/week for 8 weeks); group III (type 2 diabetic sedentary group): type 2 diabetes was induced using high-fat diet and a single intra-peritoneal injection of streptozotocin (35 mg/kg) then rats remained sedentary in their cages throughout the experimental period, and group IV (type 2 diabetic exercised group): the rats were trained by the same swimming exercise protocol after induction of type 2 diabetes.

Results: Serum irisin level significantly increased in group II compared to group I. In group III, irisin level decreased significantly compared to group I and II. A significant increase in irisin level was found in group IV compared to group III but still significantly lower than group I and II. The results also revealed a non-significant change in blood glucose, serum insulin, HOMA-IR and HOMA-β between group I and II. Blood glucose level and HOMA-IR increased significantly in group III in comparison with group I and II. However, they showed a significant decrease in group IV versus group III, but these levels remain significantly higher than those of group I and II. A non-significant change was found in serum free T3 levels among groups, while a significant decrease in serum free T4 was noticed in group III compared to group I, II and IV.

Conclusion: Type 2 diabetes was associated with a significant decrease in serum irisin level, while chronic swimming exercise training induced a significant increase in serum irisin in both healthy and type 2 diabetic rats.

Key words: Irisin, obesity, exercise, HOMA-IR.

INTRODUCTION

Type 2 diabetes is characterized by a variety of metabolic disturbances including insulin resistance, diminished glucose

uptake, elevated hepatic gluconeogenesis and mitochondrial dysfunction. At least, 25% of the incidence of type 2 diabetes mellitus is related to lack of regular physical exercise and sedentary lifestyle which were reported to cause rapid decline in insulin sensitivity, reduced insulin-stimulated glucose uptake, and glucose transporter 4 (GLUT4) protein content in human skeletal muscle (**Thyfault & Booth 2011 and Eckardt et al., 2014**).

Physical exercise is believed to have a key role in management and prevention of type 2 diabetes. Skeletal muscle contraction promotes the translocation of intracellular GLUT4 to the cell membrane resulting in an increase in skeletal muscle glucose uptake. In addition chronic exercise promotes an increase in number of GLUT4 transporters (**Cao et al., 2012**).

Skeletal muscle has been proved to act as a secretory organ that produces a variety of cytokines and peptides that are believed to mediate the beneficial effects of exercise including interleukin (IL)-6, myostatin, leukemia inhibitory factor (LIF), IL-8 and IL-15 (**Nielsen et al., 2007 and Broholm et al., 2010)** . They are refered to as “myokines”. Myokines are part of a complex communication network within the body and play a crucial role in the crosstalk between skeletal muscle and other organs, such as adipose tissue, liver and pancreas. Myokines create a systemic anti-inflammatory environment and exert an endocrine effects on visceral fat and glucose and lipid metabolism **(Pratesi et al., 2013)**. Exercise and diet have been proved to affect both adipokine and myokine levels **(Kadoglou et al., 2007 and Raschke & Eckel, 2013)**.

In this context, irisin is a novel myokine composed of 112 amino acids and has a molecular weight of 12 KDa. It was first isolated from muscle tissue. It is regulated by PPARγ coactivator 1 alpha (PGC1-α). This myokine is produced by cleavage of the product of the FNDC5 gene prior to its release into the circulation. It promotes a brown-phenotype switching in white adipose tissue, that results in enhanced thermogenesis and increased energy expenditure **(Bostr?m et al., 2012).**

Irisin is also secreted from adipose tissue. Therefore, irisin was also included in the adipokine family in addition to the myokine family **(Roca-Rivada et al., 2013)**. A possible role for irisin in glucose and lipid homeostasis was frequently investigated. It was reported that irisin signals via AMP-activated kinase (AMPK) pathway to induce glucose uptake and fatty acid oxidation in primary myocytes (**Xin et al., 2016**). Moreover, irisin was suggested to play a role in insulin action which was supported by the report that two single nucleotide polymorphisms in the *FNDC5* gene have been associated with insulin sensitivity (**Staiger et al., 2013)**. Additionally, irisin levels have been reported to correlate inversely with intrahepatic fat content in obese adults (**Zhang et al., 2013**).

Irisin was reported to increase acutely at the end of exercise (**Anastasilakis et al., 2014)**. On the other hand, some studies including systematic reviews (**Qiu et al., 2015)** and randomized control trial (**Heckstedenet al., 2013)** revealed that serum irisin levels does not increase with

chronic exercise training and may even decrease. Others reported that irisin serum levels remained stable after acute exercise or endurance training **(Czarkowska-Paczek et al., 2014**).

Type and intensity of exercise affect thyroid hormone level (**Klubo-Gwieydyinska et al., 2013**). Besides, thyroid hormones have great influence on metabolic state, thermogenesis, energy expenditure and they are reported to have a browning effect on adipose tissue as was reported for irisin. In addition thyroid hormones affect glucose homeostasis as excessive thyroid hormones are associated with increased glucose production in the liver, rapid intestinal glucose absorption, and increased insulin resistance **(Al-Geffari et al., 2013**) which may indicate a possible association between irisin and thyroid hormones in normal and diabetic subjects (**Rushala et al., 2014 and Panagiotou et al., 2016**).

In the light of the previous data about irisin and its possible role in exercise mediated improvement of metabolic disorders associated with type 2 diabetes and the conflicting data about the effect of exercise on serum irisin level, the present study was designed to evaluate serum irisin level in normal and type 2 diabetic rats and to clarify the possible effect of exercise on its level in both conditions in relation to some metabolic parameters.

MATERIALS AND METHODS

Animals: This study was conducted on 40 healthy adult male albino rats of the local strain, 8-10 weeks old with body weight 150-180 gm. They were obtained from animal house of Zagazig University. The animals were bred in the animal house and kept in steel wire cages measured 90cm x 40cm x 30cm (6-8/ cage). The rats had free access to water and chow, kept at room temperature on a natural light/ dark cycle.

The rats were accommodated to laboratory conditions for 1 weeks before starting the experimental program **(Gui et al., 2004).** The rats were randomly divided into 4 equal groups: Group **I** (control sedentary): The animals in this group were fed on standard laboratory chow diet consisted of (25.8% protein, 62.8% carbohydrates and 11.4% fat (total 12.6 KJ/g) **(Ahrén and Scheurink, 1998),** and remained sedentary with no exercise program; Group **II (**exercised group**)**: the rats were fed on standard laboratory chow diet and trained by chronic swimming exercise protocol of moderate intensity; Group **III (**diabetic sedentary group**)**: the rats remained sedentary in their cages throughout the experimental period after induction of type 2 diabetes; Group **IV** (diabetic exercised group): after induction of type 2 diabetes, the rats in this group were trained by chronic swimming exercise protocol of moderate intensity.

Induction of type 2 diabetes: After acclimation, rats in the diabetic groups were fed a high-fat chow obtained from Faculty of Veterinary Zagazig University, consisted of 16.45% protein, 25.6% carbohydrate and 58.0% fat in the form of cotton seed oil added to the laboratory chow diet (total 23.4 KJ /g) **(Cha et al., 2000).** Two weeks later, after an overnight fasting, each rat received a single intra-peritoneal injection of streptozotocin (35 mg/kg) dissolved in citrate buffer solution (pH 4.5), while rats in the non-diabetic groups (groups I and II) received a single intra peritoneal injection of

citrate buffer solution (1ml/kg) **(Okoduwa et al., 2017)**. After STZ injection, rats received 5% glucose solution as drinking water for 24 h. Diabetes was confirmed 72 h after STZ injection, using one touch glucometer. Blood samples were obtained by rat tail puncture. Rats with blood glucose levels more than 250 mg/dl were included in the study **(Coskun et al., 2004).** Death rate among rats was approximately 8% overall groups and 15% in the diabetic groups, these rats were continuously replaced.

Swimming exercise program: The rats in exercising groups were initially trained for 15 minutes/day and duration was gradually increased 5 min/day till the rats were able to perform exercise for one hour/day, which was achieved within one week to avoid water induced stress **(Lu et al., 2016)**. To achieve chronic swimming exercise, swimming rats were assigned to swim for one hour/day, 5 days/week for 8 successive weeks **(Luciano et al., 2002)**. Exercise was performed between 9:30-10:30 am. Swimming was performed in a cylindrical tank of 80 cm high , 45 cm diameter and 60 cm deep filled with tap water at 33–35 ℃ **(Lapmanee et al., 2012)**.

To achieve an exercise of moderate intensity, a small amount of detergent was added to the water while agitating the water continuously to prevent floating as the exercise intensity in the floating rat is negligible (**Fleshner et al., 1998**). When the rats are allowed to swim continuously without floating even without adding weight to the rat body or tail, the exercise is considered of moderate intensity (**Musch et al., 1990 and Fleshner et al., 1998**). At the end of each exercise session; the animals were kept to dry in a warm environment.

Anthropometric measures: The weight and length (nose to anus length) of rats were measured and BMI was calculated at the start (to determine initial BMI range) and at the end of the experiment. BMI was calculated by the equation: BMI (gm/cm^2) = body weight (gm) / $length^2$ (cm^2) , this index can be used as an indicator of obesity where the cut off value of obesity BMI is more than 0.68 gm/ cm^2 **(Novelli et al., 2007)**.

Blood sampling: After an overnight fasting, rats were sacrificed by decapitation and blood samples were obtained**.** Blood samples were allowed to clot at room temperature then centrifuged at 3000 rpm for 15 min and serum was stored at -20° C **(Gui et al., 2004)**. The animals that practiced exercise were sacrificed 48h after the end of the last training session to minimize the acute effects of exercise **(Teixeira-Lemos et al, 2011)**.

Biochemical assay:

- **Serum irisin level** was estimated by using rat double- antibody sandwich Irisin ELISA kit; (BioVendor-Laboratorni medicina, U.S.A, Cat. No.RAG018R) that was purchased from Sigma Aldrich Company according to **Teufelet et al. (2002)**.
- **Serum glucose level:** Glucose enzymatic (GOD-PAP)-liquizyme Kits (Biotech-nology, Egypt) was used as stated by **Tietz, (1995).**
- **Serum insulin level:** Rat insulin enzyme-linked immunosorbent assay kit (Product Number: RAB0904, Sigma-Aldrich Chemie GmbH, U.S.A) was used according to **Starr et al. (1978).**

- **Homeostasis model assessment of insulin resistance (HOMA-IR)** by using the formula; [HOMA-IR = insulin (?U/mL) x glucose (mg/dL) /405] (**Matthews et al., 1985 and Sun et al., 2007**).
- **Homeostasis model assessment of β cell function (HOMA-β):** HOMA-β = 20 × fasting insulin level (mU/L) / fasting glucose level (mmol/L) – 3.5 (**Matthews et al., 1985 and Sun et al., 2007**).
- **Serum total cholesterol level** by using rat cholesterol enzyme-linked immune-sorbent assay kit (Catalog Number: 2011-11-0198, Shanghai sunred biological technology, China) as described by **Flegg, (1973)** and **Allain et al. (1974)**.
- **Serum triglycerides level:** rat triglycerides enzyme-linked immune-sorbent assay kit: (Catalog Number: 2011-11-0250, Shanghai sunred biological technology, China**)** was used according to **Nagele et al. (1984)** and **Naito, (1989)**.
- **Serum high density lipoprotein cholesterol level (HDL):** Rat HDL-cholesterol enzyme-linked immune-sorbent assay kit (Catalog Number: 2011-11-0255, Shanghai sunred biological technology, China) was used according to **Warnick et al. (1983)**.
- **Serum low density lipoprotein cholesterol (LDL) level:** LDL was recorded using the following folmula: LDL=TC-HDL-TG\5 (**Friedewald et al., 1972)**.
- **Serum free T3** according to **Boscato and Stuart, (1986)** using rat Free Tri-iodothyronine (Free-T3) ELISA Kit (Biosource Europe S.A. – Rue de l'Industrie**,** 8-B- 1400 Nivelles-Belgium, Catalog No. MBS704457).
- **Serum free T4** according to **Schuurs and Van Weeman, (1977)** using rat Free thyroxine (Free-T4) ELISA Kit (Biosource Europe S.A. – Rue de l'Industrie**,** 8-B- 1400 Nivelles-Belgium, Catalog No. MBS029791).

Statistical Analysis: Data were presented as mean ± SD. Difference between the means was assayed by using analysis of variance (ANOVA) followed by post hoc test. P values less than 0.05 were considered significant. The correlations between parameters were assayed by Pearson's correlation coefficient. All statistical analyses were performed using SPSS version 18.0 program for Windows (SPSS Inc. Chicago, IL, USA).

RESULTS

The results of the present study showed a significant increase in serum irisin level in group II compared to group I ($p<0.001$). In group III irisin level decreased significantly compared to group I and II ($p<0.001$). A significant increase in irisin level was found in group IV compared to group III ($p<0.01$) but this level was significantly lower compared to group I and II ($p<0.01$ and $p<0.001$ respectively) [Table 1, Fig 1].

No significant difference in BMI was found between group I and II ($p>0.05$). In group III there was a significant increase in BMI compared to group I and II ($p<0.001$). In group IV BMI significantly decreased compared to group III ($p<0.05$), but still significantly higher than group I and II ($p<0.001$) [Table 1].

Lipid profile including TC, TG and LDL showed a non-significant difference between group I and II ($p>0.05$). However, a significant increase in HDL level was detected in group II compared with group I ($p<0.05$). Group III showed a significant increase in TC, TG and LDL ($p<0.001$) and a significant decrease in HDL versus group I ($p<0.05$) and II ($p<0.001$). Serum levels of TC, TG and LDL significantly decreased in group IV when compared to group III levels ($p<0.01$, $p<0.001$, and $p<0.001$ respectively), but still significantly higher than group I and II levels ($p<0.001$). In the same group HDL significantly increased versus group III ($p<0.05$), but no significant difference was found when compared to group I and II ($p>0.05$) [Table 1].

The results also revealed a non-significant change in blood glucose, serum insulin, HOMA-IR and HOMA-β between group I and II ($p>0.05$). In group III, blood glucose and HOMA-IR increased significantly ($p<0.001$) while serum insulin and HOMA-β decreased significantly ($p<0.01$ and $p<0.001$ respectively) in comparison with group I and II. A significant decreased in blood glucose and HOMA-IR ($p<0.001$) and a significant increase in serum insulin and HOMA-β ($p<0.05$ and $p<0.001$ respectively) was found in group IV versus group III [Table 1, Fig 2, 3, 4].

A non-significant change was found in serum free T3 levels among groups ($p>0.05$). While, a significant decrease in serum free T4 was noticed in group III compared to group I, II and IV ($p<0.01$, 0.001 and 0.001 respectively) [Table 1].

Table (1): Serum level of measured biological parameters in all studied groups.

Groups / Parameters	Group I	Group II	Group III	Group IV
BMI (g/cm)	0.55 ± 0.017	0.5 ± 0.027	0.83 ± 0.034*^{a,b}	0.74 ± 0.037*^{a,b,c}
Irisin (ng/ml)	13.42 ±1.57	19.69 ±1.25*^{a}	9.87 ± 1.39*^{a,b}	11.6 ± 1.21*^{a,b,c}
TC (mg/dl)	124 ± 25.4	122.5 ± 37.7	305.22 ±18.1*^{a,b}	250.9 ± 45*^{a,b,c}
TG (mg/dl)	110 ± 12.9	108.9 ± 27.1	245.9 ± 22.5*^{a,b}	176.2 ± 32.3*^{a,b,c}
HDL (mg/dl)	32.9 ± 5.11	37. 2 ± 2.6*^{a}	27.9 ± 4.12*^{a,b}	34.1 ± 3.11*^{c}
LDL (mg/dl)	72.6 ± 34.9	61.5 ± 12.5	232.3 ± 16.6*^{a,b}	191.9 ± 40.1*^{a,b,c}
Glucose (mg/dl)	100.8 ± 6.3	109.8 ± 4.5	255.6 ± 13.86*^{a,b}	183.96 ± 6.6*^{a,b,c}
Insulin (?IU/ml)	13.73 ± 1.26	14.03 ± 1.18	12.14 ± 1.31*^{a,b}	13.27 ± 1.16*^{c}

HOMA-IR	3.41 ± 0.019	3.8 ± 0.001	7.66 ± 0.044*[a,b]	6.027 ± 0.018*[a,b,c]
HOMA-β	45.53 ± 7.4	42.5 ± 5.23	13.59 ± 3.6*[a,b]	22.46 ± 5.12*[a,b,c]
FT3 (pg/ml)	2.96 ± 0.27	3.05 ± 0.45	2.76 ± 0.32	2.86 ± 0.22
FT4 (ng/dl)	3.38 ± 0.37	3.51± 0.24	2.85 ± 0.24*[a,b]	3.5 ± 0.51*[c]

*: significant (p<0.05); a: versus (I); b: versus (II); c: versus (III)

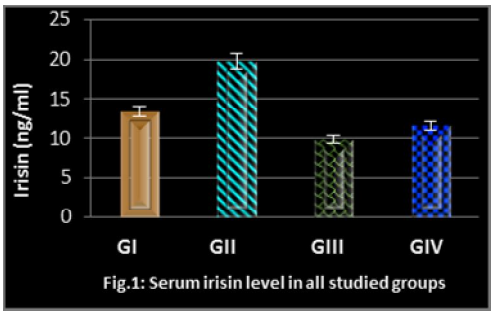

Fig.1: Serum irisin level in all studied groups

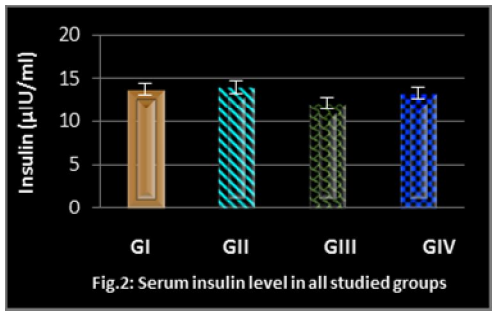

Fig.2: Serum insulin level in all studied groups

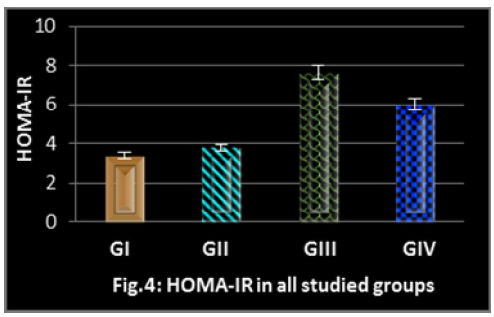

Fig.4: HOMA-IR in all studied groups

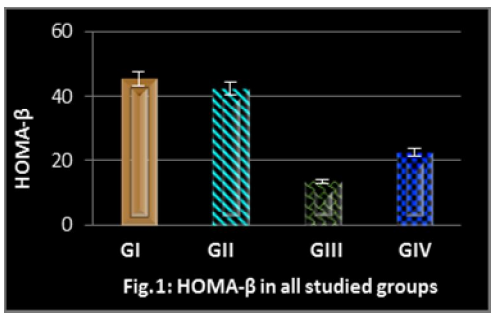

Fig.1: HOMA-β in all studied groups

The results also revealed a significant positive correlation between serum irisin levels and BMI in group I (r=0.695, p<0.05) and II (r=0.770, p<0.01) but not in group III and IV [Table 2, Fig 5, 6].

In group I serum irisin levels negatively correlated with serum HDL (r = - 0.942, p<0.001), while in group II irisin correlated positively with TC (r=0.775, p<0.01) and LDL levels (r=0.642, p<0.05) and in group III irisin correlated positively with TG (r=0.719, p<0.05) and LDL levels (r=0.681, p<0.05) and negatively with HDL (r=-0.688, p<0.05). In group IV, irisin positively correlated with serum TG (r=0.864, p<0.01) [Table 2].

Serum irisin correlated positively with FT3 in group I (r=0.730, p<0.05) and IV (r=0.642, p<0.05) [Table 2, Fig 7, 8]. No significant correlation was found between serum irisin and glucose, insulin HOMA-IR or HOMA-β in all groups [Table 2].

Table (2): Correlation between irisin and measured biological parameters.

Groups / Parametrs	Group I	Group II	Group III	Group IV
BMI (g/cm)	0.695*	0.770**	-0.207	-0.164
TC (mg/dl)	0.626	0.775**	-0.549	-0.133
TG (mg/dl)	-0.189	-0.476	0.719*	0.864**

HDL (mg/dl)	-0.942***	0.196	-0.688*	0.156
LDL (mg/dl)	0.425	0.642*	0.681*	-0.135
Glucose (mmol/L)	-0.281	0.199	-0.006	0.562
Insulin (µIu/ml)	-0.311	-0.008	-0.010	-0.018
HOMA-IR	-0.320	-0.030	-0.030	0.117
HOMA-β	0.462	0.230	0.322	0.154
FT3 (pg/ml)	0.730*	-0.306	0.301	0.642*
FT4 (ng/dl)	-0.085	0.340	-0.560	-0.399

*= p<0.05;**= p<0.001;***= p<0.001

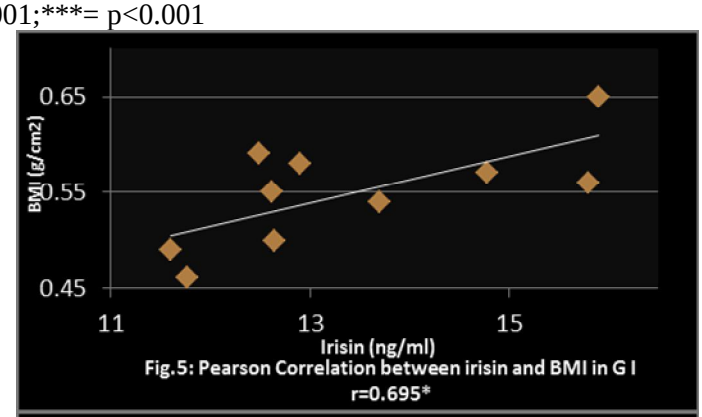

Fig.5: Pearson Correlation between irisin and BMI in G I
r=0.695*

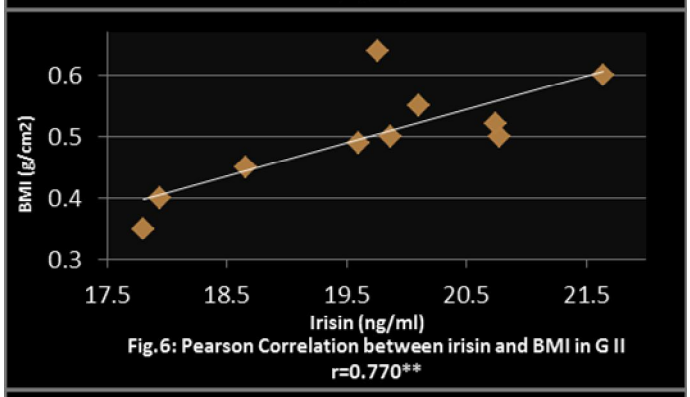

Fig.6: Pearson Correlation between irisin and BMI in G II
r=0.770**

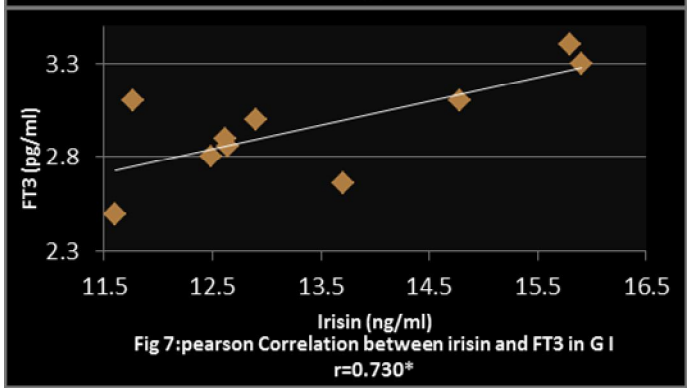

Fig 7:pearson Correlation between irisin and FT3 in G I
r=0.730*

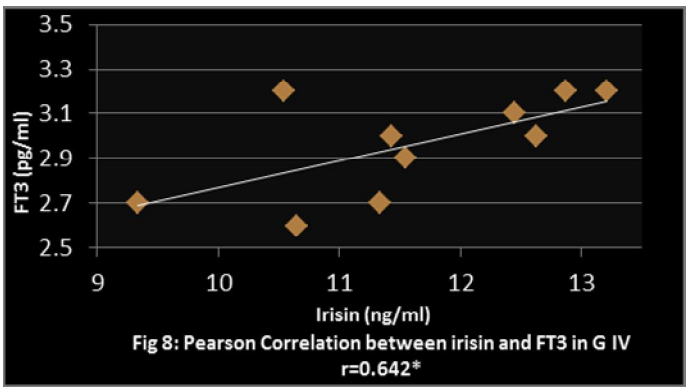

Fig 8: Pearson Correlation between irisin and FT3 in G IV
r=0.642*

DISCUSSION

Type 2 diabetes is a major health problem in different populations. Obesity and physical inactivity are referred to as main contributors in the high incidence of type 2 diabetes that increased dramatically in the recent decades with the wide spread of fast food and sedentary lifestyle (**Eckardt et al., 2014 and Leung., 2017**).

In this study, in group III, type 2 diabetic rats which received high fat diet and remained sedentary without exercise training showed a significant increase in BMI, blood glucose and HOMA-IR while serum insulin and HOMA-β significantly decreased relative to all other groups. Adverse changes in lipid profile was also recorded in this group in the form of significant increase in TG,TC, LDL and significant decrease in HDL versus group I, II, and IV. Serum irisin level in this group revealed a significant decrease versus group I (standard diet, sedentary rats). Consistent with our findings several studies revealed a reduction in circulating irisin level in type 2 diabetics compared with non-diabetic controls (**Choi et al., 2013; Guerra et al., 2013 and Liu et al., 2013**). In contrast, the study of **Fukoshima et al. (2015)** showed a significant increase in irisin levels in diabetic patient with positive correlation with HOMA-IR and suggested a compensatory role for irisin in insulin resistance. It seems that type of diabetes affects serum irisin level. An increase in irisin level was reported in type 1 diabetic patients (**Espes et al., 2015**) meanwhile, a decrease in irisin level was detected in long-term **(Liu et al., 2013; Moreno-Navarrete et al., 2013 and Assyov et al., 2016**) and newly diagnosed T2DM **(Choi et al., 2013 and Xiang et al., 2014)**, and gestational diabetes **(Zhao et al., 2015).** In addition, **Assyov et al. (2016)** demonstrated a progressive decrease in circulating irisin levels with the worsening of glucose tolerance test. Similar to these results, **Lu et al. (2016)** reported a decrease in serum irisin level in high fat diet sedentary rats relative to normal diet sedentary rats. **Liu et al. (2013)** also showed that the irisin level was high in patients with non-diabetic obesity and lower in those with type 2 diabetes mellitus. In fact these findings were a matter of debate among scientists as patients with type 2 diabetes are frequently overweight or obese and therefore an increase rather than decrease in irisin level is expected. One possible explanation of this inconsistency might be that adipose tissue and not skeletal

muscles is the main source of irisin in obese subjects without overt glucose dysregulation (**Pardo et al., 2014**) while the disturbance in glucose metabolism, insulin resistance and low grade inflammation associated with diabetes decrease irisin secretion in diabetic subjects (**Park et al., 2013**) which suggests that regulation of irisin secretion may differ between patients with and without diabetes **(Liu et al., 2013**).

Trans-membrane protein fibronectin type-III domain containing protein 5 (FNDC5) is a membrane protein that is cleaved and released as irisin **(Bostr?m et al., 2012)**. The expression of FNDC5 in skeletal muscle strictly depends on an increase in PGC1α in response to nutritional and physiological factors especially physical exercise (**Castillo-Quan, 2012**). The reduction in irisin level in group III could be a consequence of physical inactivity and obesity that induce down regulation of a about 54% of genes involved in the oxidative phosphorylation pathway in skeletal muscles including those encoding peroxisome proliferator-activated receptor (PPAR) γ coactivator 1α (PGC1α) (**Alibegovic et al., 2010**). Besides, type2 diabetes is considered a low-grade inflammation associated with overexpression of several mediators of oxidative stress and pro-inflammatory cytokines including, interleukin-1 beta (IL-1β) interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), numerous chemokines and adipo-cytokines (**Feve and Bastard, 2009**). Interestingly, IL-6 over expression was reported to reduce PGC-1*α* expression in tumor-bearing mice cells (**White et al., 2013**). Moreover, in diabetic subjects the transcription levels of IL-6 and TNFα are inversely correlated to PGC-1α levels (**Handschin et al., 2007**). Taken together, the reported increase in pro-inflammatory cytokines in type2 diabetes attenuates PGC-1*α* expression and hence decreases serum irisin level.

The results also revealed that chronic swimming exercise training induced a significant increase in serum irisin level in standard diet exercised group (II) compared to standard diet sedentary group (I). This increase in irisin level induced by chronic exercise is consistent with **Lu et al. (2016)** who detected an increase in irisin level after swimming exercise in high fat diet obese Wistar rats relative to high fat diet sedentary rats and **Winn et al. (2017)** who found an increase in obese female irisin level above base line during both modest and high intensity exercise with no differences as regard exercise intensities. Several other studies reported an increase in irisin levels by exercise in healthy subjects **(Bostrom et al., 2012; Huh et al., 2014b and Kraemer et al., 2014).** In addition, higher expression of FNDC5 mRNA was observed in muscles of patients with high aerobic performance **(Lecker et al., 2012)** and chronic exercise training **(Norheim et al., 2014).**

On the other hand, these results disagree with some authors who reported no change in the expression of muscle FNDC5 mRNA after chronic training either in muscle biopsy (**Phillips et al., 2012)** or in serum of trained subjects (**Timmons et al., 2012; Pekkala et al., 2013 and Besse-Patin et al., 2014).** Besides, irisin level was reported to be affected by type and strength of exercise. In this study chronic moderate intensity swimming exercise was conducted. In other studies irisin level was increased

after acute training but remained unchanged after chronic training in healthy adults **(Huh et al., 2012 and Loffler et al., 2015)**.

In group IV (diabetic exercised rats), chronic exercise produced a significant increase in serum irisin level versus group III (diabetic sedentary rats). Significant decrease in BMI, blood glucose and HOMA-IR with significant increase in serum insulin and HOMA-β was also recorded. Serum levels of TG, TC and LDL significantly decreased and HDL levels significantly increased indicating improvement of both lipid profile and parameters of glucose homeostasis.

The increase in irisin level in diabetic rats in response to chronic exercise can be explained by the findings of **Cao et al. (2012)** who found that chronic and acute exercise increase PGC1α mRNA expression in skeletal muscle of diabetic rats which in turn increase the expression of irisin. They also reported a reduction of blood glucose level and an increase in the expression of GLUT4 transporters and an increase in AMPK phosphorylation and expression in response to exercise in diabetic rats. GLUT4 is a glucose transporter that promotes glucose uptake in skeletal muscle and hence affects the whole-body glucose homeostasis (**Shih et al., 2009**). Both insulin and exercise increase the number of GLUT4 transporters and enhance GLUT4 translocation to skeletal muscle membrane leading to an increase in glucose uptake through divergent signaling pathways. Insulin signaling pathway involves rapid phosphorylation of insulin receptor. In contrast, exercise and muscle contraction exert its action independent of insulin receptor (**Cao et al., 2012)**. These beneficial effects of exercise are reported to be mediated at least partially by irisin as it induces improvement of glucose tolerance and glucose uptake by increasing GLUT4 translocation in diabetic skeletal muscle. Irisin also increased glucose uptake in myocytes cultured in high glucose/high fatty acid medium (**Huh et al., 2014a**). Moreover, it has been shown that irisin inhibits gluconeogenesis in diabetic liver by reducing phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) expression (**Xin et al., 2016)**. PEPCK and G6Pase are rate-limiting enzymes that control hepatic gluconeogenesis **(Leung et al 2017**). It was also revealed that, irisin reduces fat weight and serum TC and TG levels in diabetic mice, but increases acetyl CoA carboxylase-β phosphorylation in muscle tissue and uncoupling protein 1 (UCP1) expression in fat tissue. In addition, irisin increased the oxidation of fatty acid in myocytes. Irisin was reported to exert its effect on glucose and fat metabolism through AMPK activation pathway as knockdown of AMPK attenuated the effects of irisin on glucose uptake and fatty acid β-oxidation in cultured myocytes. Similarly, inhibition of AMPK by a specific inhibitor reduced the effects of irisin on PEPCK and G6Pase expression in hepatocytes (**Xin et al., 2016**).

Another possible role for irisin in improvement of glucose metabolism and insulin resistance is the anti-inflammatory effect of irisin. It was reported that irisin inhibits in a dose dependant manner the expression and function of pro-inflammatory cytokines TNFα and IL-6 in

cell medium (**Mazur-Bialy et al., 2017**). TNF-α was found to be positively correlated with the pathophysiology of insulin resistance (IR) indicating that TNF-α is a main causative factor in the development of IR. IL-6 prevents metabolism of non-oxidative glucose and decreases lipoprotein lipase leading to increases in plasma triglycerides level (**Swaroop et al., 2012 and Rehman & Akash, 2016**).

A positive correlation of irisin with BMI was found in Group I and II (standard diet groups) but not in group III and IV (diabetic groups). The previous reports about the correlation between irisin and BMI showed marked diversity. Some authors revealed a positive correlation between the expression of FNDC5 mRNA and BMI in healthy and obese subjects **(Huh et al., 2012 and Timmons et al., 2012).** In patients with anorexia nervosa, within a wide range of BMI, plasma irisin level was positively correlated with BMI, fat mass and fat free mass **(Hofmann et al., 2014).** On the other hand, **Lu et al. (2016)** reported a negative correlation of irisin with total and visceral fat mass. Other investigators found that plasma irisin level was negatively correlated with BMI in overweight subjects with Type 2 diabetics **(Moreno-Navarrete et al., 2013).** Moreover, **Winn (2017)** and his team reported an inverse association between irisin and lean body mass and suggested that the secretion of irisin from muscle and adipose tissues makes it difficult to determine its relation with different body composition parameters.

Regardless of these debates, most studies showed a positive correlation between irisin level and obesity which is in conflict with the claimed anti-obesity effect of irisin. Some authors suggested that irisin exerts a protective effect against obesity and its production from muscle and adipose tissue in obese subjects may represent an adaptive response to counteract the metabolic disturbances associated with obesity (**Pardo et al., 2014; Chen et al., 2015 and Crujeiras et al., 2015**).

In this study, irisin levels did not correlate with insulin, glucose, HOMA-IR or HOMA-β. The same was reported by **Winn et al. (2017)** who didn't find an association between plasma irisin levels and circulating glucose or insulin. This also comes in agreement with **Timmons et al. (2012)** who reported that irisin level was not correlated with glucose homeostasis. Moreover, **Pekkala et al. (2013)** did not detect any correlation between irisin and glucose homeostasis. However, Irisin was found by other investigators to be positively correlated with insulin **(Staiger et al., 2013 and Feng et al., 2015)**.

This study also showed that, in group I, serum irisin levels correlated positively with TC and negatively with HDL. In group II (standard diet, exercise) a positive correlation was found between irisin and both TC and LDL, while in group III (diabetic sedentary) irisin levels correlated positively with TG and LDL and negatively with HDL. In group IV serum irisin level correlated positively with TG. In accordance with these findings, **De La Iglesia et al. (2014)** found that the changes in irisin level paralleled the variation in the atherogenic parameters (TC, LDL-c, TC/HDL-c and

Apo B) after weight-loss therapy and suggested possible involvement of irisin in fat metabolism and lipid disorders. In obese subjects **Liu et al. (2013)** showed a positive correlation between irisin and TC. On the other hand, **Huh et al. (2012)** reported a negative association between irisin and TC while, **Wen et al. (2013)** observed a positive correlation between irisin and HDL-c, but not with TC or LDL-c. Also **Oelman et al. (2016)** detected significant inverse associations between irisin and circulating levels of total cholesterol, low-density cholesterol and triglycerides for both males and females. The marked variability in the reports about lipid profile and its association with irisin might be attributed to differences in study design, species, sample size, diet regimens or even measurement and interpretation of results.

Both thyroid hormones and irisin have metabolic effects including glucose homeostasis, increase energy expenditure and induction of adipose tissue browning which indicates a possible association between both. We found no statistically significant difference in FT3 values among groups, however an almost significant decrease (p= 0.056) in FT3 was noticed in group III (diabetic sedentary) in which FT4 level was significantly lower than all other groups. The decrease in FT4 and FT3 in this group might reflect an impact for the disturbance in glucose and lipid metabolism on thyroid function. In addition, it was reported that diabetic patients have susceptibility to different types of thyroid dysfunction, whether hypothyroidism or hyper-thyroidism with greater incidence for hypothyroidism (**Kadiyala et al., 2010 and Duntas et al., 2011**).

Slight increase in FT4 level was noticed in group II (standard diet exercise) relative to group I (standard diet sedentary) but didn't reach statistical significance. A significant increase in serum level of FT4 was found in group IV (diabetic exercised) when compared with group III (diabetic sedentary). The increase of FT4 in response to exercise is in agreement with previous studies such as **Fortunato et al. (2008)** who reported an increase in T3 and T4 in adult rats immediately after exercise. Moreover, **Bansal et al. (2015)** reported that regular exercise increased T3 and T4 and decreased TSH levels in human subjects. In contrast, other studies demonstrated a reduction of T3, T4 and TSH in male athletes subjected to intense physical exercise for 1 week proportionate to the degree of training (**Hackney et al., 2012**). This contrast might be explained by the high intensity and short duration exercise protocol in the later study, while in the current study, a moderate intensity swimming exercise protocol was conducted for 8 weeks. Besides, species difference may play a role.

We also found in this study a positive correlation between irisin level and FT3 in group I and group IV and this come in line with previous studies that demonstrated a positive correlation between irisin and free thyroxine **(Ruchala et al., 2014).** This positive correlation is supported by previous findings reported that TSH infusion increased mRNA expressions of PGC-1α **(Martinez et al., 2016)**. In addition, exercise increases skeletal muscle expression of type 2 deiodinase enzyme (D2) through a β-adrenergic receptor-dependent mechanism. D2 accelerates the conversion of T4 to T3

within myocytes which then participate in the induction of PGC-1α during exercise. This effect was absent in mouse subjected to D2 gene inactivation in skeletal muscle fibres (**Bocco et al., 2016**). The increase in PGC-1α in turn increases irisin expression as mentioned before which can explain this positive association.

However, our results disagreed with **Ellefsen et al. (2014)** who found no association between irisin level and thyroid hormones after 12-week strength training in untrained women. Variation in exercise intensity, sex and species may be a cause of this conflict. Also, **Panagiotou et al. (2016)** reported no association between serun irisin and TSH, free thyroxine and free triidothyronine, in either euthyroid and/or subclinical hyperthyroid subgroups and suggested that irisin induced metabolic effects are independent of thyroid hormonal axis. Unlike our study, the previous study was conducted without exercise protocol. Exercise has great influence on irisin and thyroid hormone levels. So, different results are expected.

CONCLUSION

A significant decrease in serum irisin level was found in type 2 diabetic rats. Chronic swimming exercise training induced an increase in serum irisin level in both normal and type 2 diabetic rats with significant improvement of glucose homeostasis parameters and lipid profile indicating that irisin might be one of the main contributors in the exercise mediated beneficial effects and put a spotlight on irisin as a possible future therapy for type 2 diabetes and its related metabolic disorders.

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تأثير ممارسة التمارين الرياضية الهوائية طويلة المدة علي مستوي الأيريزين في مصل الجرذان المصابة بداء السكري من النوع الثاني

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خلفية البحث: تلعب التمارين الرياضية و الغذاء دورا محوريا في علاج داء السكري من النوع الثاني. هذا، و تفرز العضلات مواد متنوعة تسمي ميوكاين و التي يعتقد أنها مسؤلة عن التأثير الإيجابي للتمارين الرياضية. و في هذا السياق، يفرز بيبتيد الأيريزين من العضلات بتأثير التمارين الرياضية و أيضا يفرز من الخلايا الدهنية، و لذلك يمكن إعتباره ميوكاين و أديبوكاين. ولقد أوضحت الدراسات أن للأيريزين تأثير علي توازن الجلوكوز و التمثيل الغذائي للدهون.

الهدف من البحث: دراسة تأثير تمارين السباحة الهوائية طويلة المدة علي مستوي الأيريزين في مصل الجرذان المصابة بداء السكري من النوع الثاني و دراسة علاقة هذه المستويات ببعض معاملات التمثيل الغذائي.

مواد و طرق البحث: تم إستخدام 40 من ذكور الجرذان البيضاء البالغة، و تم تقسيمها إلي أربع مجموعات متساوية كالتالي:

- **المجموعة الأولي:** و فيها تتناول الجرذان غذاء طبيعيا قياسيا ولا تخضع للتمارين الرياضية.
- **المجموعة الثانية:** و فيها تتناول الجرذان غذاء طبيعيا قياسيا و تخضع لتمارين السباحة المزمنة متوسطة الشدة.
- **المجموعة الثالثه:** الجرذان المصابة بداء السكري من النوع الثاني ولا تخضع للتمارين الرياضية
- **المجموعة الرابعة:** الجرذان المصابة بداء السكري من النوع الثاني و تخضع لتمارين السباحة المزمنة متوسطة الشدة.

النتائج: حدثت زيادة ذات دلالة إحصائية في مستوي الأيريزين في مصل الدم في المجموعة الثانية مقارنة بالمجموعة الأولي. أما في المجموعة الثالثة، فحدث نقص ذو دلالة إحصائية في مستوي الأيريزين مقارنة بالمجموعة الأولي و الثانية. كما أوضحت النتائج حدوث زيادة ذات دلالة إحصائية في مستوي الأيريزين في المجموعة الرابعة مقارنة بالمجموعة الثالثة، و لكنه لا يزال أقل من مستواه في المجموعتين الأولي والثانية.

الخلاصة: داء السكري من النوع الثاني يصاحبه نقص في مستوي الأيريزين بينما التمارين الرياضية الهوائية طويلة المدة تحدث زيادة في مستوي الأيريزين في كل من الجرذان الطبيعية والمصابة بداء السكري من النوع الثاني.