

Effect of muscle exercise on metabolic disturbance induced by ovariectomy alone or with diabetes mellitus in rats; targeting the role of PGC-1 α and FNDC5 expression

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Keywords

- Muscle exercise
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Abstract

This study investigated the possible role of regular moderate exercise on type 2 diabetes mellitus in ovariectomized rats.

Methods: 50 female albino rats divided into 5 groups; control sham operated sedentary (CSS), ovariectomized sedentary (OS), ovariectomized trained (OT), diabetic ovariectomized sedentary (DOS) and diabetic ovariectomized trained (DOT).

At the end of the experiment body mass index and body weight gain percentage were calculated then serum was used for determination of levels of irisin, glucose, insulin, lipid profile, tumor necrosis factor-alpha (TNF- α), interleukin 1 beta (IL1 β), interleukin 4 (IL4), interleukin 10 (IL10), malondialdehyde (MDA) and reduced glutathione (GSH). Furthermore, gastrocnemius muscle biopsies were taken for determination of peroxisome proliferator-activated receptor gamma co-activator 1-alpha (PGC-1 α) and fibronectin type III domain-containing protein 5 (FNDC5) relative gene expressions. **Results:** induction of diabetes after bilateral ovariectomy further exacerbated the disturbed metabolic, inflammatory and oxidative statuses, indicated by increased BMI, body weight gain percentage, total cholesterol, LDL, glucose, insulin, HOMA-IR, TNF- α , IL-1 β and MDA levels, and decreased HDL, IL4, IL10 and GSH levels, in addition to decreased Irisin level and PGC-1 α and FNDC5 expressions in OS and DOS groups compared to CSS and OS groups respectively. On the other hand, regular moderate exercise significantly alleviated all these disturbances, in concomitance with improvement of the irisin level and PGC-1 α and FNDC5 expressions in OT and DOT groups compared to OS and DOS groups respectively. **Conclusion:** muscle exercise improved the metabolic, inflammatory and oxidative disturbances induced by estrogen deficiency and aggravated by diabetes, possibly via increasing serum irisin.

INTRODUCTION

Estrogen plays a key protective role against obesity and metabolic diseases. Estrogen is an important regulator of many metabolic processes, such as glucose and lipid metabolism, energy expenditure, body weight, and adipose tissue distribution [1]. Estrogen deficiency that occurs physiologically after menopause is associated with many problematic adverse effects, such as loss of muscle mass [2], and metabolic disturbances including insulin resistance, and increased fat mass [3], with the possibility of developing obesity that increases the risk of cardiovascular diseases and type 2 diabetes mellitus [4], thus it is considered as a very important public health issue [3].

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder characterized by insulin resistance, elevated blood glucose level and disturbed lipid metabolism [5]. Many experimental studies on animals induce T2DM by feeding rodents a high-fat diet (HFD). However, female rodents showed protection against this HFD-induced T2DM, this protection was suggested to be due to the endogenous estrogen [6]. Thus, ovariectomy is often performed in rodents to simulate the postmenopausal state in women [7]. Previous studies reported that ovariectomy in female rodents results in manifestations similar to that occur in postmenopausal women, like increased body weight, fat mass [6], insulin resistance and blood glucose level [8]. Moreover, combined ovariectomy and HFD produces further increased weight gain, and diminished protection from T2DM, suggesting that estrogen loss could increase the risk of T2DM in female rodents, especially when combined with a HFD [6].

Physical activity is considered as a key component in the management of T2DM. It has been suggested that participation in regular physical exercise could prevent or delay occurrence of T2DM in non-diabetics [9], or improve blood glucose level and reduce complications in people with T2DM [10], as exercise may improve the β -cells function within the pancreas [11].

Irisin is firstly identified in 2012 as a novel myokine secreted mainly from skeletal muscles following exercise or exposure to cold [12], by hydrolysis of fibronectin type III domain-containing protein 5 (FNDC5), which was reported to be a peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1 α)-dependent hormone [13]. PGC-1 α could regulate the secretion of irisin, either directly by increasing FNDC5 hydrolysis to irisin [14], or indirectly through increasing FNDC5 mRNA expression of in skeletal muscle, [15].

Moreover, the effect of exercise on serum irisin level is still under research. Some authors reported elevation in the serum irisin levels after training for 3 weeks [16]. However, others found that exercise training does not affect the circulating irisin level or skeletal muscle FNDC-5 [17].

So, the purpose of this study was to investigate the possible protective role of regular moderate muscle exercise, mediated by irisin signaling, on metabolic disturbance exerted by estrogen deficiency and exaggerated by T2DM in ovariectomized HFD-induced diabetic rats which was used as a model for postmenopausal diabetic women.

2. Material and methods

2.1. Animals and study design:

The current study was performed at Faculty of Medicine, Tanta University, from June 2019 to December 2019, and all experiments were conducted according to guidelines of the Ethical Committee of Medical Research, of Tanta Faculty of Medicine, Egypt. Fifty female Wistar rats, of same age (about 7 weeks), weighing 180-200g, were purchased from the Experimental Animal House of Tanta Faculty of Science. The rats were kept in special cages (5 rats per cage), at an environmental temperature of 22–25°C with a 12h light/12h dark cycle, with free access to water and food.

The rats were randomly categorized into 5 equal groups, 10 rats each.

Group 1: Control sham operated sedentary (CSS) group, subjected to simulated surgery.

Group 2: Ovariectomized sedentary (OS) group: subjected to bilateral ovariectomy.

Group 3: Ovariectomized trained (OT) group, subjected to exercise protocol after bilateral ovariectomy.

Group 4: Diabetic ovariectomized sedentary (DOS) group: diabetes was induced 1 week after bilateral ovariectomy.

Group 5: Diabetic ovariectomized trained (DOT) group, subjected to exercise protocol after bilateral ovariectomy and induction of diabetes.

2.2. Surgical procedure:

At the start of the experiment, rats were weighted (initial weight) then **CSS** group underwent a sham operation (ovaries were manipulated but not removed) while all other groups underwent a bilateral ovariectomy according to the method described by Abdel-Sater

and Mansour [18]. Rats were allowed to recover for one week in individual cages, during which they received anti-inflammatory and antibiotic drugs as described by Silva et al., [19].

2.3. Induction of type 2 diabetes mellitus (T2DM):

After the recovery period, T2DM was induced in **DOS** and **DOT** groups by feeding rats with a high-fat diet (HFD; prepared by adding sucrose and ram tail fat to the basal diet in a ratio of 20% and 10% (w/w) respectively) for 4 weeks, followed by a single intraperitoneal (i.p.) injection of 30 mg/kg freshly prepared streptozotocin (STZ; purchased from Sigma Chemical Co. St. Louis, USA). The STZ low dose exhibited a minor trauma to pancreatic β cells that simulate the condition of chronic insulin resistance [20]. 6 h after STZ administration, rats received 10% dextrose solution for the next 24 hours, to prevent the STZ induced hypoglycemia. Fasting blood glucose was measured in rats' tails blood using a glucometer (Accutrend Plus; Roche, Mannheim, Germany) and only rats with fasting blood glucose exceeding 200 mg/dl were included in the experiments and continued to feed HFD for another 6 weeks [21], keeping the number of rats 10 in such groups, then those in **DOT** group were subjected to a swimming exercise.

2.4. Exercise protocol:

Rats in the **OT** and **DOT** groups were subjected to a moderate swimming exercise without a load, in a water-filled (40 cm depth) barrel (70 cm diameter \times 70 cm height). The temperature of the water was kept at $35 \pm 2^\circ\text{C}$, so as not to affect the core temperature. Firstly, rats underwent adaptation training for one week; started by swimming for 10-15 min/day, extended by 10-15

min every day until the rats could swim for 60 min/day. Then the exercise protocol was followed as 60 min swimming/day, 5 days/week for 8 successive weeks [22].

2.5. Parameters:

At the end of the experiment (18 weeks after recovery from surgery in CSS, OS and DOS groups, or just after finalizing the exercise protocol in OT and DOT groups) rats were weighted again (final weight) to calculate the *Anthropometric measures*, then all rats had been fasted for 16-18 hours and blood samples were collected under anaesthesia, centrifuged at 3000 rpm for 20 min. at room temperature. The obtained serum was kept at -20°C to be used for *Biochemical analysis*. Also gastrocnemius muscle biopsies were taken for Quantitative measurement of peroxisome proliferator-activated receptor gamma co-activator 1-alpha (PGC-1 α) and fibronectin type III domain-containing protein 5 (FNDC5) relative genes expression by quantitative real-time reverse transcription PCR (RTPCR).

2.5.1. Anthropometric measures:

Rats were anaesthetized by a single i.p. injection of sodium pentobarbital (40 mg/kg) [23], to measure the body weight (using a digital balance), and the rat length (nose to anus length) [24] then the body weight gain was calculated (final weight-initial weight) to get the body weight gain percentage (BWG%) using this equation: (Weight Gain/Previous Weight) \times 100%, and the body mass index (BMI) was calculated using this equation: Body weight (kg)/length² (m²) [25].

2.5.2. Biochemical analysis:

The obtained serum was used to measure:

2.5.2.1. Serum glucose, insulin and insulin resistance:

Serum glucose level was measured using colorimetric assay kits (Biodiagnostic Chemical Company, Giza, Egypt) according to manufacturers' instructions, and serum insulin was measured by Rat Insulin ELISA kit (MyBioSource, Inc., USA) according to manufacturers' instructions. Insulin resistance (IR) was calculated using the equation of homeostasis model assessment of IR (HOMA-IR) as described by Matthews et al., [26], where;

$$HOMA - IR = \frac{Glucose (mg/dl) \times insulin(\mu IU/ml)}{405}$$

2.5.2.2. Serum lipid profile:

Serum triglycerides (TG), total cholesterol (TC), and high density lipoprotein (HDL-C) were determined using Biodiagnostic kits (Giza, Egypt) following the manufacturers' instructions. While, LDL-C Concentration was calculated using Friedewald equation [27], where; LDL-C = TC - (HDL-C + TG/5).

2.5.2.3. Serum oxidative stress markers:

Malondialdehyde (MDA) and reduced glutathione (GSH) levels were determined in serum using their colorimetric assay kits (MyBioSource, Inc., USA), according to the manufacturers' instructions

2.5.2.4. Serum Cytokines:

Serum TNF- α , IL1 β , IL4 and IL10 levels were determined using commercial ELISA kits, provided by MyBioSource, (Inc., USA), according to the manufacturers' instructions

2.5.2.5. Serum irisin: was measured using rat Irisin ELISA kit; (MyBioSource, Inc., USA) following the manufacturers' instructions.

2.5.3. Quantitative measurement of PGC-1 α and FNDC5 relative genes expression by quantitative real-time reverse transcription PCR (RT-PCR)

2.5.3.1. RNA extraction:

Total RNA was extracted from 30 mg of the left rat gastrocnemius muscle biopsies using total RNA Purification Kit following the manufacturer protocol (Thermo Scientific, Fermentas, #K0731). RNA concentration and purity were measured using a Nano drop spectrophotometer (Implen, USA). RNA was then preserved at -80 °C.

2.5.3.2. cDNA synthesis:

RNA was reverse transcribed according to the manufacturer's instructions using Revert Aid H Minus Reverse Transcriptase kit (Thermo Scientific, Fermentas, #EP0451).

2.5.3.3. Real-time quantitative PCR:

PCR reactions were performed using Thermo Scientific Maxima SYBR Green Master Mix (Thermo scientific, USA, # K0221) following the manufacturer's instructions using a Step One Plus real time thermal cycler (Applied Biosystems, Life technology, USA) as follows; initial denaturation at 95°C for 10 minutes followed by 40 cycles with denaturation at 95°C for 15 seconds, annealing at 60°C for 30 seconds and extension at 72°C for 30 seconds.

Primer sequence specific for PGC-1 α gene were designed according to a recently published study [28] as follows; forward 5'-TAGGCCAGGTACGACAGCTA-3' and reverse 5'-TTCTGTCCGCGTTGTGTCAG-3'. Primers for the housekeeping gene β -actin, which was used as a reference gene, were as follows: forward 5'-CATTGCTGACAGGATGCAGAAGG-3' and

reverse 5'-GGAGTGACAGGTGGAAGGTCGT-3'.

Primer sequence specific for FNDC5 gene were designed according to a recently published study [29] as follows: forward 5'-GTCTCCCACCACCATCTT-3' and reverse 5'-TCTGTCTCTGAGTGTAGCCTTAGC-3'.

Primers for GAPDH, which was used as a reference gene, were as follows: forward 5'-GTGCCAGCCTCGTCTCATAG-3' and reverse 5'-GACTGTGCCGTTGAACTTGC-3'.

The cycle threshold (Ct) values were calculated for target gene and the reference gene, and relative gene expression was determined using 2- $\Delta\Delta$ Ct method [30].

2.6. Statistical analysis:

Collected data were statistically analyzed using the SPSS software version 23.0, by one-way ANOVA followed by Tukey's to determine the significance between more than two groups. Data were expressed as mean \pm SD. P values < 0.05 were considered significant.

3. Results

3.1. Effect of muscle exercise on anthropometric measures

The present study showed that ovariectomy induced a significant increase in the BMI and BWG% that was further increased after induction of diabetes, as shown in OS and DOS groups compared to CSS and OS groups respectively. Groups that underwent exercise for 8 weeks showed significant improvement in the BMI and BWG% as detected in OT and DOT groups compared to OS and DOS groups respectively, figure 1.

3.2. Effect of muscle exercise on lipid profile, serum glucose, insulin and HOMA-IR:

Table 1 showed that serum levels of triglyceride, total cholesterol, LDL-C, insulin and glucose together with HOMA-IR were significantly increased, while HDL-C was significantly decreased after ovariectomy and more after induction of diabetes as shown in OS and DOS groups compared to CSS and OS groups respectively. Groups that underwent exercise for 8 weeks showed significant improvement in all these changes as detected in OT and DOT groups compared to OS and DOS groups respectively.

3.3. Effect of muscle exercise on serum oxidative stress markers and cytokines:

The serum levels of GSH, IL4 and IL10 were significantly decreased, while serum levels of MDA, TNF- α and IL-1 β , were significantly increased, in OS versus CSS group as well as in

DOS versus OS group. After 8 weeks of regular exercise a significant increase in GSH, IL4 and IL10 and decrease in MDA, TNF- α and IL-1 β were detected in OT and DOT groups versus OS and DOS groups respectively, table 2.

3.4. Effect of muscle exercise on serum irisin level and PGC-1 α and FNDC5 relative genes expression

It was noticed that serum level of irisin (table 2 and figure 2A) as well as the expression of both PGC-1 α (figure 2B) and FNDC5 (figure 2C), were significantly decreased in OS and further decreased in DOS groups as compared to CSS and OS groups respectively. While, regular moderate swimming exercise for 8 weeks significantly increased the serum irisin level as well as the expression of both PGC-1 α and FNDC5, as detected in OT and DOT groups compared to OS and DOS groups respectively.

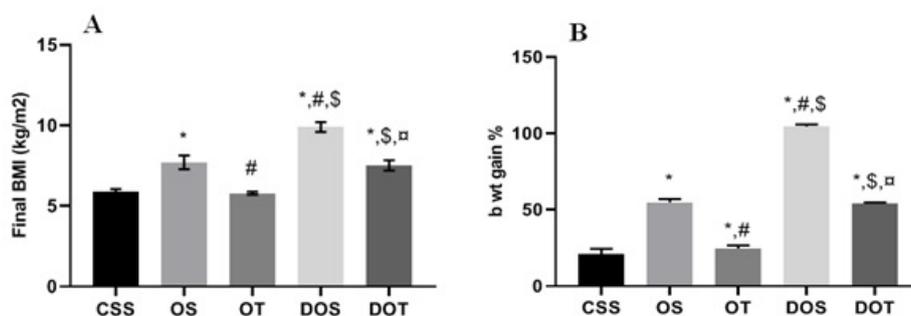


Figure 1: Final BMI (A) and body weight gain % (B) for all groups.

Values are expressed as mean \pm SD (n=10). Significance of differences ($P < 0.05$) is illustrated as *versus CSS group; # versus OS; \$ versus OT; □ versus DOS

Table 1: lipid profile, serum glucose, insulin and HOMA-IR for all groups.

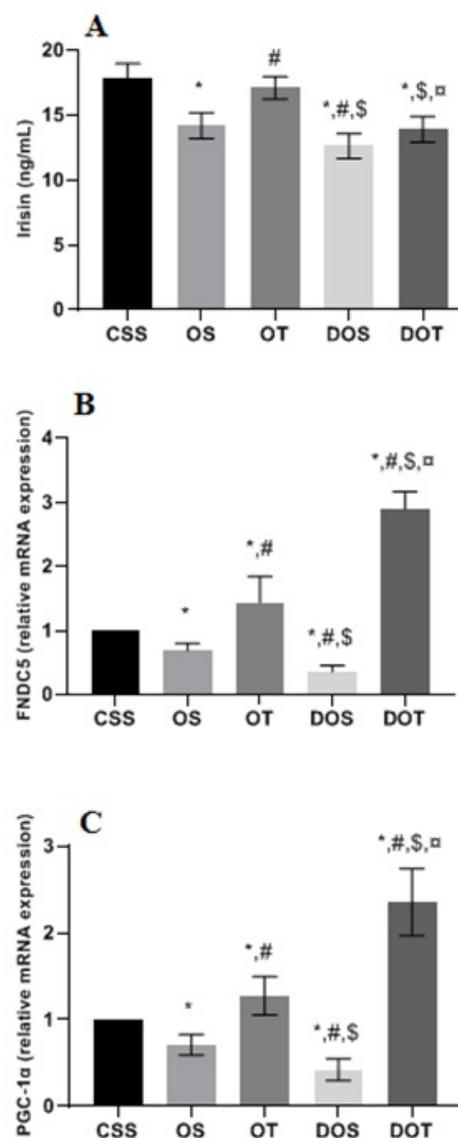
Parameters	CSS	OS	OT	DOS	DOT
TG (mg/dl)	45.20 \pm 5.88	59.74 \pm 2.35*	50.06 \pm 3.66#	104.4 \pm 4.54*#,\$	65.03 \pm 2.73*#,\$,□
Total cholesterol (mg/dl)	87.49 \pm 7.38	105.7 \pm 7.55*	90.13 \pm 7.32#	182.2 \pm 21.57*#,\$	110.1 \pm 10.54*#,\$,□
HDL-C (mg/dl)	53.11 \pm 3.63	46.13 \pm 3.03*	51.01 \pm 3.51#	31.03 \pm 2.76*#,\$	38.45 \pm 2.99*#,\$,□
LDL-C (mg/dl)	24.47 \pm 1.39	41.52 \pm 4.96*	26.18 \pm 3.31#	124.6 \pm 2.69*#,\$	53.24 \pm 2.97*#,\$,□
Glucose (mg/dl)	87.70 \pm 5.31	103.5 \pm 3.03*	90.30 \pm 6.99#	250.2 \pm 10.01*#,\$	109.8 \pm 5.33*#,\$,□
Insulin (μ IU/ml)	4.57 \pm 0.67	13.30 \pm 0.72*	6.25 \pm 0.89*#	16.28 \pm 1.12*#,\$	13.99 \pm 0.87*#,\$,□
HOMA-IR	1.04 \pm 0.05	3.46 \pm 0.18*	1.36 \pm 0.05#	7.60 \pm 0.62*#,\$	2.97 \pm 0.20*#,\$,□

Values are expressed as mean \pm SD (n=10). Significance of differences ($P < 0.05$) is illustrated as *versus CSS group; # versus OS; \$ versus OT; □ versus DOS. TG; triglycerides, HDL-C; high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol.

Table 2: oxidative stress markers, cytokines and irisin levels for all groups.

Parameters	CSS	OS	OT	DOS	DOT
GSH ($\mu\text{mol/L}$)	73.87 \pm 3.04	61.47 \pm 6.83*	70.22 \pm 4.82#	43.28 \pm 6.69*,#,\$	56.44 \pm 6.37*,\$,\square
MDA ($\mu\text{mol/L}$)	48.13 \pm 2.60	62.34 \pm 4.05*	52.53 \pm 3.45*,#	90.25 \pm 2.63*,#,\$	57.25 \pm 2.84*,#,\$,\square
TNF- α (pg/mL)	35.30 \pm 2.86	48.78 \pm 2.64*	37.93 \pm 2.00#	70.52 \pm 2.78*,#,\$	52.01 \pm 2.80*,\$,\square
IL-1 β (pg/mL)	20.68 \pm 3.66	42.09 \pm 3.31*	20.33 \pm 3.66#	93.37 \pm 6.79*,#,\$	29.55 \pm 3.50*,#,\$,\square
IL4 (pg/mL)	275.8 \pm 12.72	222.1 \pm 11.81*	239.7 \pm 19.38*,#	116.4 \pm 11.10*,#,\$	198.6 \pm 8.45*,#,\$,\square
IL10 (pg/mL)	111.8 \pm 6.94	70.05 \pm 3.62*	86.79 \pm 7.80*,#	25.18 \pm 2.72*,#,\$	52.59 \pm 3.68*,#,\$,\square
Irisin (ng/mL)	17.85 \pm 1.13	14.20 \pm 1.00*	17.11 \pm 0.87#	12.62 \pm 0.95*,#,\$	13.91 \pm 1.00*,\$,\square

Values are expressed as mean \pm SD (n=10). Significance of differences ($P < 0.05$) is illustrated as *versus CSS group; # versus OS; \$ versus OT; \square versus DOS. GSH: reduced glutathione, MDA; malondialdehyde, TNF; tumor necrosis factor, IL; interleukin.

**Figure 2:** serum irisin level (A), PGC-1 α (B), and FNDC5 (C) relative genes expression for all groups.

Values are expressed as mean \pm SD (n=10). Significance of differences ($P < 0.05$) is illustrated as *versus CSS group; # versus OS; \$ versus OT; \square versus DOS.

4. Discussion

In this work, the regular moderate exercise exerted clear beneficial effects on metabolic disturbance induced by ovariectomy as well as ovariectomy associated with type 2 diabetes mellitus, which may be previously investigated but by a different method. Such beneficial effects could be mediated by the myokine irisin through increasing the gene expression of its precursor FNDC5, as explained in the following discussion;

The present study revealed a significant increase in body weight gain percentage after ovariectomy. In agreement with previous studies [31], this finding could be attributed to the decreased physical activity and energy expenditure following ovariectomy [32]. Moreover, further increase was detected after induction of diabetes in DOS rats, in accordance with findings of other studies, explained by fat accumulation secondary to utilization of a high fat diet, together with low energy expenditure [21].

It is well known that ovariectomy is associated with general changes in the metabolism [8] and diabetes also produces disturbed lipid and carbohydrate metabolism [33]. Consistently, the present study showed disturbed lipid and carbohydrate metabolism in OS rats exaggerated by induction of diabetes in DOS rats, as indicated by significant elevation of serum levels of TG, TC and LDL-C, as well as glucose and insulin together with increased HOMA-IR value in DOS more than OS rats.

In harmony, previous studies reported impaired lipid and/or carbohydrate metabolism in diabetic [20], ovariectomized [21], and diabetic ovariectomized rats [5] as well as, in postmenopausal women [34].

The deficiency of ovarian hormones after ovariectomy is the keystone of these metabolic changes [25]; this could be attributed to the associated dyslipidemia, impaired glucose tolerance and insulin resistance [35]. Moreover, Mooradian, [36] reported an enhanced lipolysis in insulin-resistant adipocytes.

Furthermore; the present study showed an exaggerated oxidative stress state in DOS more than OS rats manifested by higher MDA and lower GSH levels. This oxidative stress could be attributed to the loss of antioxidant effect of estrogen [37]. In addition, diabetes is well-known to induce a general oxidative stress status, through disturbance of the mitochondrial electron transport chain [38], and alteration of the total antioxidant capacity [39]. Also, it was suggested that oxidative stress could increase insulin resistance and accelerate diabetic complications in diabetic rats [40].

The present study revealed an ovariectomy-induced inflammatory state, exaggerated by induction of diabetes as indicated by increased pro-inflammatory cytokines (TNF- α and IL1 β) and decreased anti-inflammatory cytokines (IL4 and IL10) in DOS more than OS rats. This inflammatory state might play a role in insulin resistance as mentioned by Minihane et al., [41] who suggested that insulin signaling is impaired by pro-inflammatory mediators released from adipocytes with further induction of insulin resistance. This could explain the increased HOMA-IR in DOS more than OS group.

Furthermore, a strong relation was detected between metabolic syndrome and oxidative stress where hyperglycemia, excess TNF- α and oxidized LDL, all increase the generation of reactive

oxygen species, resulting in accumulation of the lipid peroxidation end products and increased oxidative stress [42]

After 8 weeks of swimming exercise, a significant improvement was detected in metabolic profile, inflammatory and oxidative stress markers of OT and DOT groups compared with OS and DOS groups respectively. In agreement with these findings; previous studies detected an exercise-induced improvement of metabolic profile [17], inflammatory state [42] and oxidative stress markers [9].

These effects may be mediated by myokines, including irisin [42], in addition, the exercise-induced increase in energy consumption could explain the improvement of metabolic profile [13]. Also, the reduction of BMI in OT and DOT group could be attributed to the increased fat metabolism and energy consumption with their weight-lowering action [13].

Interestingly, the serum irisin level was reduced in DOS more than OS rats, but a significant increase in serum irisin level was detected in the OT and DOT groups relative to the OS and DOS groups respectively, suggesting that estrogen deficiency and T2DM decreased while swimming exercise increased the serum irisin level.

These findings came in accordance with studies that revealed reduction of serum irisin levels in postmenopausal women [43]. This could be explained by the sarcopenia accompanying estrogen deficiency [44], as confirmed by Huh et al., [45] who reported a significant positive correlation between estrogen and circulating irisin and suggested that estrogen may increase irisin secretion directly or through increasing muscle

mass via its anabolic effect [45]. Additionally, previous studies reported a decrease in serum irisin level in type 2 diabetic patients, corrected after insulin therapy [46]. On the other hand, exercise has been reported to significantly increase the serum irisin level in HFD rats [42].

The previously discussed beneficial effects of exercise were supposed to be mediated by irisin hormone [42], as a significant negative correlations were detected between serum irisin level versus BMI [47], visceral fat content, [48], waist circumference [43], serum total cholesterol, triglyceride, LDL [13] serum glucose, insulin, HOMA-IR, TNF- α and MDA [42].

The exercise induced-improvements in obesity and glucose homeostasis in both OT and DOT rats could be explained by the concomitant increase in irisin level which was reported to enhance the total energy consumption and fat oxidation [13], thus considered as a target for prevention and treatment of obesity [16]. Also, Bourlier et al., [49] referred the role of irisin in alleviation of metabolic disorders to the improved aerobic capacity and glucose metabolism in the whole body.

Huh et al., [50] reported an exercise-dependent increase in irisin level in human beings. Similarly, Boström et al. [16] reported an exercise dependent increase in irisin expression. On the other hand, Huh et al., [45], reported a reduction in serum irisin level related to decreased muscle FNDC5 expression secondary to the post-surgical weight loss.

In agreement with our findings, Norheim et al., [51] observed an exercise-induced significant increase of FNDC5 expression in skeletal muscle in diabetic overweight subjects. Moreover, PGC-1 α , was reported to be strongly correlated with

adaptations induced by exercise training [52]. The exercise-induced PGC-1 α expression in skeletal muscle, promoted FNDC5 expression, and subsequently synthesis of irisin [53].

Interestingly, the increased PGC-1 α expression after exercise is mediated by several molecular pathways. The activation of adenosine monophosphate- protein kinase (AMPK), calcium/calmodulin-dependent protein kinase (CaMK), p38 mitogen-activated protein kinase (MAPK), and calcineurin A (CnA) signaling cascades are well-known regulators of PGC-1 α expression in skeletal muscle [54].

Limitations of the study: presence of control groups that were trained alone, diabetic alone or only received high fat diet and measurement of more parameters as serum estrogen level for example would allow better understand of the underlining mechanisms. But, to reduce the cost of this study, we relied on the results of previous researches in such points.

In conclusion, muscle exercise might have a protective role against metabolic, inflammatory and oxidative stress disturbances induced by estrogen deficiency and aggravated by type 2 diabetes mellitus, this may be attributed to the increased serum irisin secondary to increased PGC-1 α and FNDC5 gene expression in skeletal muscle. Further investigations may be required about other factors that may affect irisin level and the effect of exogenous irisin on postmenopausal metabolic disturbances and their complications.

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Conflict of interest:

The authors declare that they have no conflict of interest.

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