Bull. Egypt. Soc. Physiol. Sci. 40 (1): 96-112

Bull. of Egyp. Soc. Physiol. Sci.

(Official Journal of Egyptian Society for Physiological Sciences)

(pISSN: 1110-0842; eISSN: 2356-9514)

Potential impact of exercise versus Irisin on Hypertension, and Visceral Adiposity in a Rat Model of type 2 Diabetes Mellitus

[1]Fatma M. Lebda, [1]Mona A. Ahmed, [1]Mona K. Shawky, [2]Walaa Baher, [1]Yasmine Sabry

From

[1]Medical Physiology Department, Ain Shams Faculty of Medicine, Cairo, Egypt

[2]Histology Department, Ain Shams Faculty of Medicine, Cairo, Egypt

Received: 17 July 2019
Accepted: 11 August 2019
Available online: 1 Jan 2020

Keywords

- Diabetes
- Visceral adiposity
- Hypertension
- Exercise
- Irisin

Abstract

Background: Type 2 diabetes mellitus (T2DM) is associated with obesity, insulin resistance, and hypertension. Exercise may play important role in combating obesity and diabetic complications. Irisin is a newly discovered exercise-induced myokine. Its role in mediating the beneficial effects of exercise is questionable. **Aim:** The present study was performed to reveal the beneficial effects of moderate-intensity exercise on diabetic-induced visceral obesity, and hypertension in a rat model of T2DM, as well as the potential role of irisin relative to exercise and the mechanisms-involved. **Materials and methods:** Rats were allocated into 4 groups; control, type 2 diabetes mellitus (T2DM), exercise-T2DM and irisin-T2DM groups. Body weight (BW), body mass index (BMI), perirenal fat (PF), systolic (SBP), diastolic (DBP), and mean blood pressures (MBP), fasting blood glucose (FBG), insulin, nitrite, and HOMA-insulin resistance (IR) and histology of adipose tissue were determined. **Results:** Exercise attenuated the adverse effects of T2DM, whereas PF, PF index, serum nitrite, plasma insulin, and HOMA-IR comparable to controls; however, FBG, SBP, DBP and MBP still significantly higher. Partial browning of white adipose tissue demonstrated. Irisin-T2DM rats showed a remarkable effect compared to exercise intervention documented by a reduction in BW, BMI, PF, PF index, DBP, FBG, and insulin, with increase in nitrite, and complete browning of adipose tissue. **Conclusion** It is concluded that exercise and irisin treatment can improve visceral adiposity and hypertension; however, the protective effect of irisin is more obvious. These data suggest irisin as a potential new strategy to combat obesity and hypertension in diabetic patients.

Corresponding author: Yasmine Sabry, Medical Physiology Department, Ain Shams Faculty of Medicine, Cairo, Egypt, Email: yassogss@hotmail.com; Mob: +201101188555

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is characterized by insufficient insulin secretion from the β-cells of the pancreatic islets, coupled with insulin resistance (1). Insulin resistance and T2DM are closely associated with obesity and ectopic fat accumulation (2), as well as hypertension (3).

The accumulation of visceral fat plays an important role in the pathogenesis of hypertension, insulin resistance, and abnormal secretion of adipokines (4).

Animal models of T2DM have been proved to be useful to study the impact of, and to find a new therapy for, the disease. Earlier study has demonstrated that a combination of high-fat diet with low dose of STZ can be effectively used to generate a rat model that simulates the natural history and metabolic derangements of the common T2DM in humans (5).

It has been shown that exercise increases insulin sensitivity in tissues, enhances glucose uptake into cells, thus improving glycemic control, and reducing the metabolic risk factors associated with T2DM (6), in addition to reducing abdominal adiposity (7), by enhanced adipose tissue lipolysis (8), and to reversing the diabetes-induced hypertension (9).

Irisin is a new myokine, formed from the cleavage of the transmembrane protein fibronectin type III domain-containing protein 5 (Fndc5) (10). Previous study indicated that circulating serum irisin levels were decreased in T2DM subjects (11). However, prolonged aerobic exercise induced transient increase in irisin concentrations in young men and women, suggesting that this form of moderate exercise may be helpful in improving fat metabolism (12).

Irisin targets white adipocytes to induce browning, activating thermogenesis to increase energy expenditure, and as well promotes pancreatic β-cell proliferation and improves glucose tolerance (13). In addition, administration of irisin reduced blood pressure in both control and spontaneously hypertensive rats and dilated mesenteric artery rings through ATP-sensitive potassium channels (14). However, irisin was inversely associated with insulin sensitivity (15).

While the protective role of exercise is recognized in diabetes, the available data concerning the underlying mechanisms are inconclusive. Identification of the antihypertensive and anti-adiposity effects of irisin in diabetic patients is an area of active, ongoing research that may help to determine therapeutic targets for diabetes mellitus.

Therefore, this study was designed to investigate the therapeutic, preventive effects of swimming intervention on the visceral adiposity and hypertension in a rat model of T2DM; meanwhile, to assess and confirm the potential role of irisin relative to exercise training and to explore the mechanisms-involved.

Materials and methods

Chemicals and drugs

Streptozotocin (STZ) was purchased from Sigma-Aldrich, Inc.; USA. Citrate buffer was obtained from Morgan Specialty Chemicals, Egypt. Irisin-Fc fusion, recombinant, was supplied as white powder by Phoenix Pharmaceuticals, Inc, USA, and it was reconstituted in sterile water, to obtain irisin solution of 100 ng/ml.

Experimental Animals

The present study was carried out on 86 adult male Wistar rats, initially weighing 170-230 g. Rats were purchased from and housed in the Animal House of Medical Ain Shams Research Institute (MASRI), under standard conditions of boarding. Rats were left for a period of 2 weeks for acclimatization before starting the experiments. The rats were placed in plastic cages (5-6 rats/cage) with free access to diet that was introduced daily at 8:00 a.m. and water ad libitum, and were maintained at room temperature (22±1 °C) with 12 h light/dark cycle. The ethical guidelines of Ain Shams University for the care and use of Laboratory animals were adopted. Further, the Ain Shams Faculty of Medicine Ethical Committee approval was obtained.

The rats were allocated into two dietary regimens by feeding either high fat diet or standard (control) diet. The high fat diet consisted of 57.3% fat, 24.6% CHO, and 14.1% protein, with a total caloric value of 670.5/100 g dry food, while the control diet was composed of 5.85% fat, 67.3% carbohydrate, and 23% protein, with a total caloric value of 413.85/100 g dry food (16).

Induction of Type 2 diabetic rat model

Type 2 diabetes was induced in rats by high-fat diet and low dose of STZ, according to the method of (17). Following 3 weeks of HFD intake, rats were injected, i.p. with a single dose of STZ (30mg/kg, BW) dissolved in 1 mL of 0.05M citrate buffer. After one week, rats having fasting blood glucose levels ≥ 200 mg/dl were considered diabetics and were included in the study.

Exercise Protocol

The model of exercise used in the present study was moderate-intensity swimming. It was carried out in an individual swimming system consisting of polyvinyl chloride (PVC) pipes (20 cm wide and 50 cm long) that were placed inside the tank (18). This protocol comprised of 30 min swimming, with a workload equivalent to 5% of the animal's body weight, 5 times/week for 4 weeks.

Study design:

Experimental Protocol:

Rats were randomly divided into four groups. Control Group (n= 20): Rats fed with control diet, and received a single intraperitoneal (i.p.) injection of citrate buffer, equivalent to that given with STZ. The diabetic rats were randomly allocated into three experimental groups: T2DM untreated group (n=23); T2DM-Ex group (n=23): One week after STZ injection, diabetic rats were subjected to moderate-intensity swim exercise for 30 min, 5 days/week for 4 weeks ,and continued feeding with HFD till end of the experimental period; and T2DM-irisin group (n=20): One week after STZ injection, diabetic rats were subjected to i.p. injection of irisin solution in a dose of 150 µL/rat, 6 days/week for 4 weeks, and continued feeding with HFD till end of the experimental period

Experimental Procedures

All rats were subjected to measurement of body weight, BMI, and arterial blood pressure, just before start (initial reading) and at end (final reading) of the experimental period. Blood pressure was measured, using the non-invasive small animal tail blood pressure system

(NIBP200A, Biopac systems Inc; USA). Three measurements for blood pressure were recorded and mean value was calculated. BMI was calculated as follows: BMI= Body weight (gm)/ length (cm^2) (19). The % change of BW, BMI and blood pressure was calculated as follows: % change = $\frac{Final-Initial}{Initial}$ x 100. The fasting blood glucose level was determined by using Gluco-Star 2 blood glucose monitoring system (TaiDoc Technology Corporation, Taiwan).

On the day of sacrifice, overnight fasted rats, were anesthetized by i.p. injection of pentobarbitone (40 mg/kg, BW). Blood samples were collected from the abdominal aorta. The separated plasma was used for determination of plasma insulin level using the kit supplied by Perfect Ease Biotech (Beijing) Co., Ltd., China, while the separated serum was used for determination of serum nitrite level using the kit supplied by supplied by Bio-diagnostic, Egypt, and performed according to the manufacturer's instructions.

The perirenal fat was excised, blotted dry with filter paper and weighed. Perirenal fat index was calculated as follows: perirenal fat index= perirenal fat weight (gm)/body weight (gm) x 100% (20). Insulin resistance was calculated using the homeostasis model assessment of insulin resistance (HOMA-IR) as follows: HOMA-IR = fasting plasma insulin (µLU/mL) x fasting plasma glucose (mmol/L) /22.5 (21)

Histological studies

Histology Analysis of inguinal adipose tissue: Small pieces of inguinal adipose tissue were dissected out and fixed with 10% formalin solution and embedded in paraffin. Sections (8 µm) were cut and stained with hematoxylin and eosin (H&E). For microscopic examination, a microscope (Leica, DM2500) was used, and the images were taken at Histology and Cell Biology Department- Faculty of Medicine- Ain Shams University, using a Canon EOS 1100D Digital SLR camera at 10 (ocular)× 40 (object lens) magnification.

Statistical analysis:

All the results are expressed as mean ±SEM. The one way ANOVA test was used to assess the statistical significance of the difference of a parametric variable between more than two study groups. The Mann Whitney Test (U test) was used to assess the statistical significance of the difference of a non-parametric variable between two study groups. A value of $P \leq 0.05$ was considered statistically significant. All statistical data and significance were performed by using SPSS (Statistical Program for Social Science) statistical package (SPSS Inc.) version 22.0.

Results:

Changes in body weight (BW), body mass index (BMI), and perirenal fat

As shown in Tables 1, Type 2 diabetic rats had a significant reduction ($P \leq 0.001$) in the final BW, % BW change ($P \leq 0.001$), final BMI ($P \leq 0.001$), and % BMI change ($P \leq 0.05$) compared to those in the controls. In diabetic rats subjected to moderate exercise, final BW and the % of BW change were higher, being statistically significant only for final BW ($P \leq 0.05$), in comparison to type 2 diabetic rats, but both of them were significantly less ($P \leq 0.001$) than their corresponding values in

the control group. However, both final BMI and % BMI change were non-significantly different compared to T2DM group, but they were significantly decreased compared to control group (P≤0.001 and P≤0.05, respectively).

Treating diabetic rats with irisin resulted in non-significant changes in final BW and % change of BW compared to T2DM group. On the other hand, final body weight significantly reduced (P≤0.05), while % BW change showed body weight loss but was statistically non-significant compared to the T2DM-Ex group. However, both final BW and % BW change were significantly reduced when compared to control group (P≤0.001). Also, there is significant reduction in final BMI and % BMI change compare to type 2 DM group (P≤0.05) and to control group (P≤0.001), being statistically significant only for final BMI (P≤0.05) when compared to exercise group.

PF and PFI were significantly increased in T2DM rats, compared to controls (P≤0.001). Exercise exhibited a significant diminution in PF and PFI in T2DM-Ex group, in comparison toT2DM group (P≤0.005, P≤0.001, respectively), becoming comparable to the corresponding values in the control group. Treatment with irisin resulted in significant decrease in PF and PFI compared to T2DM group (P≤0.001), and T2DM-Ex group (P≤0.005, P≤0.02, respectively). Values of PF and PFI were, also, less than that of the control rats, although non-significantly different.

Table 1: Changes in the body weight (BW), body mass index (BMI), and their percent changes (% change), perirenal fat weight (PF) and perirenal fat index (PFI) in the different studied groups.

	BW (g)			BMI (g/cm^2)			PF (g)	PFI (g/100g BW)
Group	**Initial**	**Final**	**% change**	**Initial**	**Final**	**% change**		
Control	210.58 ±4.49 (19)	261.11 ±6.59 (19)	24.58 ±3.39 (19)	0.54 ±0.01 (19)	0.56 ±0.01 (19)	4.95 ±3.33 (19)	1.29 ±0.17 (20)	0.48 ±0.05 (20)
T2DM	208.44 ±3.83 (18)	206.67 ±4.92 (18)	-0.54 ±2.42 (18)	0.53 ±0.01 (18)	0.51 ±0.01 (18)	-4.16 ±2.31 (18)	2.07 ±0.10 (23)	1.03 ±0.05 (23)
P	NS	<0.001	<0.001	NS	<0.001	<0.05	<0.001	<0.001
T2DM-Ex	219.82 ±6.12 (17)	225.82 ±5.57 (17)	3.54 ±3.04 (17)	0.53 ±0.02 (17)	0.50 ±0.01 (17)	-4.56 ±2.82 (17)	1.52 ±0.15 (23)	0.62 ±0.06 (23)
P	NS	<0.001	<0.001	NS	<0.001	<0.05	NS	NS
P*	NS	<0.05	NS	NS	NS	NS	<0.005	<0.001
T2DM-irisin	208.00 ±3.52 (17)	205.18 ±7.17 (17)	-1.19 ±3.31 (17)	0.53 ±0.01 (17)	0.47 ±0.01 (17)	-11.17 ±2.39 (17)	0.91 ±0.11 (20)	0.44 ±0.05 (20)
P	NS	<0.001	<0.001	NS	<0.001	<0.001	NS	NS
P*	NS	NS	NS	NS	<0.05	<0.05	<0.001	<0.001
P**	NS	<0.05	NS	NS	<0.05	NS	<0.005	<0.02

Results are expressed as mean ±SEM. In the parenthesis is the number of rats. NS: Not significant, P: Significance from control group, calculated by LSD at P≤ 0.05, P*: Significance from type 2 diabetes (T2DM) group, calculated by LSD at P≤ 0.05, P**: Significance from type 2 diabetes-exercise (T2DM-Ex) group, calculated by LSD at P≤ 0.05, Significance of % change was calculated by Mann-Whitney U test at P≤0.05.

Changes in systolic blood pressure, diastolic blood pressure, mean blood pressure, and serum levels of nitrite

As presented in Table 2, there was a significant elevation in the final SBP, final DBP, and final MAP, and their % changes (P≤0.001), in contrast to a significant fall in serum nitrite level (P≤0.001) in T2DM group as compared to control group.

Moderate exercise training resulted in a significant fall in final SBP and % SBP change (P≤0.001 and P≤0.02, respectively), final DBP and % DBP change (P≤0.001), and final MAP and % MAP change (P≤0.001 and P≤0.005, respectively), as compared to T2DM group. But all of these parameters were still significantly higher (P≤0.001), as compared to control group. Serum nitrite was significantly increased (P≤0.001) in T2DM-Ex group versus T2DM group, reaching level insignificantly different from the control group.

Treatment with irisin induced a significant drop in final SBP, final DBP, final MAP and their % changes in T2DM-Irisin group as compared to T2DM group (P≤0.001); however, only final DBP and % DBP were significantly decreased (P≤0.001), in comparison to T2DM-Ex group. On the other hand, final SBP, final MAP and their % changes (P≤0.001) and final DBP (P≤0.005), were still significantly increased, compared to the corresponding values in control group. Serum nitrite was significantly increased in T2DM-Irisin group compared to T2DM group (P≤0.001) and T2DM-Ex group (P≤0.005), reaching level insignificantly different from the control group.

Table 2: Changes in the systolic blood pressure (SBP), diastolic blood pressure (DBP), mean blood pressure (MBP) and their percent changes (% change), and serum nitrite level in the different studied groups.

	SBP (mmHg)			DBP (mmHg)			MBP (mmHg)			Nitrite (µmol/L)
Group	**Initial**	**Final**	**% change**	**Initial**	**Final**	**% change**	**Initial**	**Final**	**% change**	
Control	123.20 ±0.83 (20)	119.85 ±1.13 (20)	-2.66 ±1.01 (20)	21.98 ±2.14 (8)	74.45 ±0.75 (20)	-2.84 ±0.92 (20)	107.70 ±0.66 (20)	104.72 ±0.93 (20)	-2.74 ±0.82 (20)	21.98 ±2.14 (8)
T2 DM	121.52 ±1.15 (23)	144.22 ±1.52 (23)	18.96 ±0.79 (23)	9.90 ±0.97 (13)	91.48 ±1.42 (23)	19.86 ±1.04 (23)	106.49 ±0.94 (23)	126.64 ±1.32 (23)	19.13 ±1.29 (23)	9.90 ±0.97 (13)
P	NS	<0.001	<0.001	<0.001	<0.001	<0.001	NS	<0.001	<0.001	<0.001
T2 DM-Ex	120.30 ±1.85 (20)	132.65 ±0.93 (20)	10.87 ±2.17 (20)	18.65 ±1.41 (13)	84.80 ±0.61 (20)	9.85 ±1.46 (20)	106.02 ±1.56 (20)	116.70 ±0.59 (20)	10.59 ±1.91 (20)	18.65 ±1.41 (13)
P	NS	<0.001	<0.001	NS	<0.001	<0.001	NS	<0.001	<0.001	NS
P*	NS	<0.001	<0.02	<0.001	<0.001	<0.001	NS	<0.001	<0.005	<0.001
T2 DM-irisin	120.50 ±1.35 (18)	132.94 ±0.65 (18)	10.54 ±1.20 (18)	24.04 ±0.87 (13)	78.50 ±0.84 (18)	0.12 ±1.59 (18)	106.52 ±0.99 (18)	114.79 ±0.61 (18)	7.91 ±1.03 (18)	24.04 ±0.87 (13)
P	NS	<0.001	<0.001	NS	<0.005	NS	NS	<0.001	<0.001	NS
P*	NS	<0.001	<0.001	<0.001	<0.001	<0.001	NS	<0.001	<0.001	<0.001
P**	NS	NS	NS	<0.005	<0.001	<0.001	NS	NS	NS	<0.005

Results are expressed as mean ±SEM. In the parenthesis is the number of rats. NS: Not significant, P: Significance from control group, calculated by LSD at P≤ 0.05, P*: Significance from type 2 diabetes (T2DM) group, calculated by LSD at P≤ 0.05, P**: Significance from type 2 diabetes-exercise (T2DM-Ex) group, calculated by LSD at P≤ 0.05, Significance of % change was calculated by Mann-Whitney U test at P≤0.05.

Changes in fasting blood glucose, plasma insulin, and HOMA-IR

As displayed in Figure 1, T2DM group showed significant increase in FBG, plasma insulin level and HOMA-IR as compared to control group ***(P≤0.001).*** T2DM-Ex group exhibited a significant decrease in FBG, plasma insulin and HOMA-IR compared to T2DM group ***(P≤0.001)***; however, only FBG was still significantly increased ***(P≤0.001)*** compared to the controls.

In T2DM-Irisin group, FBG, plasma insulin and HOMA-IR showed significant reduction compared to T2DM group ***(P≤0.001).*** While, FBG ***(P≤0.005)*** and plasma insulin ***(P≤0.05)*** were significantly decreased, HOMA-IR was insignificantly changed in comparison to T2DM-Ex group. However, all these parameters were non-significantly different compared to the corresponding values in the controls.

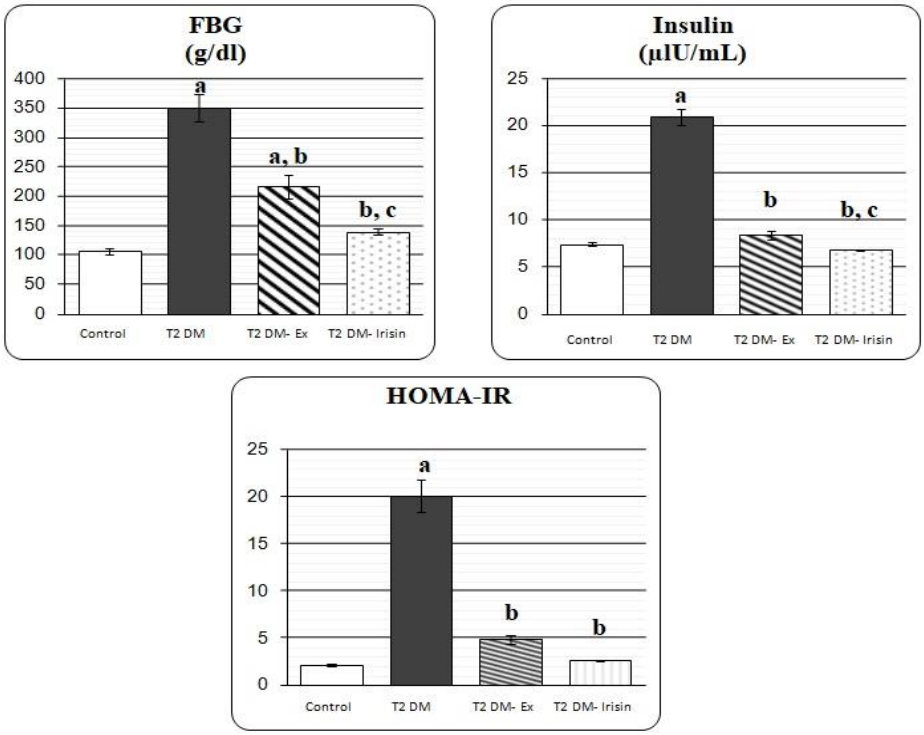

Figure 1: Fasting blood glucose (FBG), plasma insulin, and homeostasis model assessment of insulin resistance (HOMA-IR) in the different studied groups.

Results are expressed as mean ±SEM, **a:** Significance from control group, calculated by LSD at P≤ 0.05, **b:** Significance from type 2 diabetes (T2DM) group, calculated by LSD at P≤ 0.05, **c:** Significance from type 2 diabetes-exercise (T2DM-Ex) group, calculated by LSD at P≤ 0.05.

Histological analysis of inguinal adipose tissue:

Examination of H&E stained inguinal adipose tissue sections in the control group showed that it was formed entirely of white adipose tissue (WAT). The adipocytes were polygonal, unilocular and having signet ring appearance. In each unilocular adipocyte, the triglycerides are stored in a single large droplet occupying most of the cell space, displacing and flattening the nucleus against the cell membrane. The cytoplasm appeared as very thin rim surrounding the lipid droplet (Figure 2a). In the T2DM the inguinal adipose tissue was of the white type comparable to the control. However, large amount of collagen fibers and inflammatory cells appeared in between the adipocytes (Figure 2b). In the T2DM-Ex, although most of the adipocytes were of the unilocular type, many brown-like adipocytes (Beige cells) appeared between them. These cells were having multiple lipid droplets in their cytoplasm (multilocular) and a centrally located nuclei (Figure 2c). In the T2DM-Irisin group, the inguinal adipose tissue showed almost complete browning. Most adipocyte were of the multilocular type. Their sizes were smaller than the unilocular adipocytes and their cytoplasm contained multiple, variable sizes lipid droplets and central nuclei. Only few adipocytes of the unilocular type were detected in between (Figure 2d).

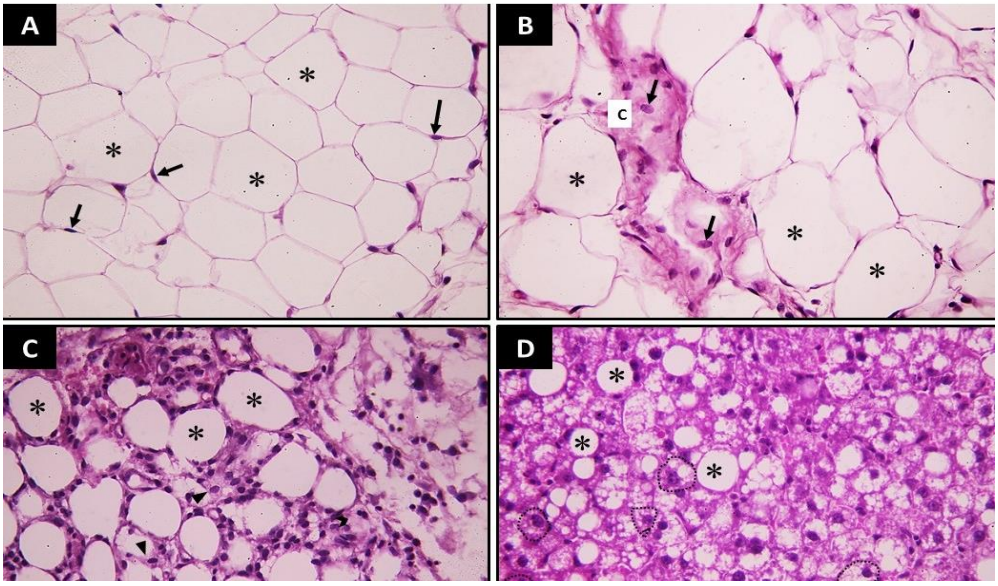

Figure 2: A photomicrograph of inguinal adipose tissue; **(A): Control:** The adipose tissue is of the white type. It is formed of unilocular adipocytes, polygonal in shape and having signet ring appearance. Each adipocyte contains a single large lipid droplet (∗) displacing and flattening the nucleus (↑) against the cell membrane. The cytoplasm appears as a thin rim around the lipid droplet. **(B): T2DM group**: white adipose tissue formed of unilocular adipocytes (∗) comparable to the control. In between the adipocytes, large amount of collagen fibers (c) and the inflammatory cells (↑) are seen. **(C): T2DM-Ex group:** Most of the adipocytes are of the unilocular type (∗). In between the unilocular adipocytes, many brown-like (beige cells) adipocytes having central nuclei and many small lipid droplets in their cytoplasm (▲). **(D):** T2DM-**Irisin group:** the adipose tissue is formed mainly of small size, multilocular adipocytes comparable to brown adipose tissue. Their cytoplasm contains multiple lipid droplets of variable sizes and centrally located nuclei (dotted cells). Few adipocytes of the unilocular type (∗) are interspersed in between the multilocular adipocytes. (H&E X400)

Discussion

Type 2 diabetes is often associated with cardiovascular disease risk factors including obesity, high blood pressure, dyslipidemia, and lack of physical activity (22). In the current study, a combination of high fat-diet and low dose of streptozotocin treatment was used to generate a rat model that simulatesthe metabolic derangements that occur in type 2 diabetes in humans (17). Thus, this animal model of type 2 diabetes could be suitable for evaluating the potential therapeutic effects of moderate-intensity exercise training versus irisin hormone.

In the present study, T2DM rats exhibited a significant decrease in the final BW, and the final BMI, and their % change, as well as, increased PF weight and PFI compared to control group. The observed body weight loss in T2DM rats may be due to skeletal muscle atrophy caused by the diabetes-induced-metabolic derangement, motor end-plate degeneration, and impairment of myocyte protein synthesis (57), and by oxidative stress (58).

While exercise training (30 min, 5 days/week for 4 weeks) resulted in successful attenuation of the increased PF weight and PF index in T2DM, which became comparable to the corresponding values in the control group. Moreover, the values of final BW, final BMI, and their % change in exercising diabetic rats were significantly lower than that in the control group.

The present post exercise results seem to be consistent with the histological study of the adipocytes in the inguinal adipose tissue that displayed many brown-like adipocytes (Beige cells), having multiple lipid droplets in their cytoplasm compared to that in T2DM which showed unilocular white type with large amount of collagen fibers and inflammatory cells. Collectively, these data indicate browning of some adipocytes with expected increase in energy expenditure, and suggest that exercise training can potentially be helpful in combating obesity in diabetic patients.

Similarly, Ibanez et al. (23), and Johnson et al. (24) detected significant decrease in visceral fat without significant weight loss in exercising T2DM patients, while Piao et al. (25) found significant decrease in visceral fat weight and improved body composition in exercising diabetic obese rats. Likewise, Mohammadi et al. (26) reported significant decrease in visceral fat, and increase in the lean mass in exercising diabetic patients.

Piao et al. (25) reported that exercise treatment improves composition of both lean body mass and fat body mass, while Pesta et al. (27) indicated that chronic exercise increases muscle mass and strength via induction of muscle hypertrophy and neuromuscular remodeling. Interestingly in the present study, there is significant increase in the final BW of exercising diabetic rats compared to that of T2DM group, which may be attributed to the increase in muscle mass and the decrease in diabetes-induced muscle atrophy and weight loss. Further, the significant reduction in the BW, BMI and their % change compared to the control values, as well as, the significant decrease in the PF and PFI compared to T2DM rats, being comparable to control group; collectively, are suggestive of a better body composition through decreasing the fat mass and increasing the muscle mass.

In the current study, rats treated with irisin (150 µL/rat, 6 days/week for 4 weeks) showed that the final BW, BMI, PF and PFI were significantly lower than that in exercising diabetic rats. A similar result has been reported by a number of investigators; Zhu et al. (28) indicated that irisin injection caused significant decrease in BW in HFD/STZ diabetic mice, while Hou et al. (29) found that treatment of obese mice with irisin reduced body weight and visceral fat. Also, Mo et al. (30) reported that irisin-transgenic mice had a significantly decreased mass of visceral and subcutaneous adipose tissue, and decreased adipocyte size than wild-type mice.

The results of the present study obviously denote better effect for irisin relative to exercise on metabolic derangement and body obesity associated with diabetes, and emphasize that irisin can effectively abrogate the diabetes-evoked obesity, documented by the significant reduction in PF and PFI, compared to both T2DM and exercising T2DM rats, reaching levels match the normal control values. In addition, most adipocytes in the inguinal adipose tissue were small in size and of the multilocular brown-like type, clearly reveal the conversion of white adipocytes into the brown type; consistent with the present findings, irisin treatment in obese mice reduced the size of adipocytes in subcutaneous adipose tissue and stimulates lipolysis, resulting in less lipid accumulation and smaller adipocytes (31).

Several previous studies reported that irisin therapy successfully prevented the induction of obesity in rats as evident by significant weight reduction in rat subjected to irisin injection and on high fat diet (32). It has been suggested that irisin can potentially prevent obesity and associated type 2 diabetes in mice by stimulating expression of visceral adipose tissue browning-specific genes via the p38 MAPK and ERK pathways (13). Earlier studies reported that irisin acts on white adipose cells in culture and in vivo to stimulate UCP1 expression and a broad program of brown fat-like development, increasing in energy expenditure in mice with no changes in movement or food intake (10).

In the view of histological analysis in the current study, that demonstrated partial browning of white adipose tissue in the exercise group; Lehnig and Stanford (33) reported that, exercise may contribute to “beiging” of WAT, a process which is also referred to as the “browning” of AT. During this process white adipocytes change and resemble the characteristics of brown adipocytes in the brown adipose tissue. Moreover, reduction of beige adipocytes resulted in the development of obesity and insulin resistance in mice; this implies that these cells may play a role in the regulation of systemic energy metabolism (34).

Intrestingly, irisin resulted in complete browning of inguinal white adipose tissue. Zhang et al. (13) demonstrated that irisin induced functional brown-like adipocyte phenotype through promotion of brown fat-specific gene expression in white adipose tissue.

On the other hand, progressive decline in insulin action (insulin resistance), and hyperinsulinemia are the main pathogeneses in type 2 diabetes mellitus (35). Herein, the rats with T2DM exhibited a significant rise in fasting blood glucose, plasma insulin levels and increased HOMA-IR when compared to control group. Exercise training, in the current study, improved

the hyperglycemic state in T2DM. These results seem to be consistent with the previously reported significant decrease in fasting blood glucose and insulin levels, and improved HOMA-IR and insulin sensitivity in T2DM rodents enrolled in exercise regimen (36), and in the exercise treated diabetic patients (37).

It is noteworthy that moderate-intensity exercise training; herein, significantly reversed the elevated values of FBG, plasma insulin and HOMA-IR in T2DM rats. Interestingly, plasma insulin and HOMA-IR reached levels non-significantly different from that in the control rats; in contrast, FBG was still significantly higher than control values. These findings document the important role of regular exercise training in ameliorating the development of hyperglycemia and insulin resistance in this T2DM rat model and confirm the efficacy of exercise intervention in glucose homeostasis.

Previously, it has been reported that aerobic exercise increases muscle glucose uptake up to five fold through insulin-independent mechanisms (38), linked to increase in AMPK which via many steps enhances GLUT-4 vesicle translocation and glucose uptake into the cell, increasing muscle glycogen storage following exercise training (39). In addition, exercise stimulates glycogen synthase activity, thus increasing insulin sensitivity (40), and could improve glycemic control via the enhancement of the β-cell mass and function through decreasing glucolipotoxicity and reducing β-cell apoptosis (41).

Similar to exercise treatment, irisin supplementation, in the present study, caused significant decrease in FBG, plasma insulin level, and HOMA-IR compared to T2DM group. These findings are in line with previous reports found that irisin treatment reduces the risk of insulin resistance as revealed by significant decrease in insulin and glucose levels in fat mice fed with HFD (13, 31), and in adult obese rats (42).

It is worth noting that after irisin supplementation, in the current study, there was more reduction in FBG and plasma insulin than that after exercise training, reaching levels non-significantly different from the corresponding control values. These results, obviously, reveal that irisin is more effective than exercise in abrogating the glycemic derangement associated with diabetes, indicated by the reversed hyperglycemia and the improved insulin resistance, suggesting that irisin strongly contributes to glycemic control in rats with T2DM.

According to more recent experimental reports, irisin exhibits therapeutic potential in insulin resistance and type 2 diabetes mellitus by stimulating browning of white adipose tissue, promoting glucose uptake in skeletal muscle and heart, and improving pancreatic β cell function, through the activation of intracellular signaling pathways (43). The conversion of white adipocytes to brown adipocytes, observed in this study, leads to increase in energy expenditure and thermogenesis with subsequent improvement of insulin sensitivity, reductions in body weight, and improved glucose tolerance in mice (31, 44). Also, irisin has anti-apoptotic actions on pancreatic beta-cells (45), and increases glucose uptake in skeletal muscles and mature adipocytes via upregulation of GLUT4 expression (46).

On the other side, T2DM rats exhibited a significant rise in final SBP, DBP, and MBP, as well as their % changes, in contrast to a significant

drop in the serum nitrite level when compared to control group; however, these changes were reversed by aerobic exercise training, in the current study. These beneficial effects of exercise on diabetic-induced hypertension have been documented by previous clinical studies; type 2 diabetic patients, following aerobic exercise programme, demonstrated a significant reduction in SBP compared to controls (47), while hypertensive diabetic patients showed a decrease in blood pressure and an increase in heart rate variability after regular exercise training (48).

Previous studies documented increased irisin levels after exercise (49), while other one demonstrated decreased circulating serum irisin levels in T2DM (11). This may draw attention to the potential role of irisin in, partly, mediating the beneficial effects of exercise in diabetics. Interestingly, in the present study, the protective and the preventive role of irisin in T2DM was proved, as it was not only able to reverse the diabetic-induced hypertension and to improve nitrite levels in T2DM rats, but it, also, significantly reduced diastolic blood pressure and elevated serum nitrite levels more than that in exercising diabetic rats. Based on these results, the present study hypothesized that irisin is more effective in lowering blood pressure compared to exercise.

As in exercise, the blood lowering effect of irisin is mediated mainly by decreasing total peripheral resistance. Irisin might improve arterial relaxation, and promote vasodilatation, thus decreasing peripheral resistance, which can be achieved via several mechanisms including: phosphorylation of eNOS, stimulating NO release and subsequent vasodilatation of both large conduit and resistance arteries and ultimately decreased blood pressure (50), or mitigating the advanced glycation end products (AGE)-induced inflammation and endothelial dysfunction in cultured human umbilical vein endothelial cells (51). These reports are supported by the increased nitrite levels, biomarker of NO release, and the decreased hyperglycemia detected in the present study after exercise intervention and irisin injection. Taken together, it can be assumed that moderate exercise training and irisin attenuate the diabetes-evoked hypertension, possibly by improving glycemic control and/or by abrogating the the impaired NO generation in diabetic rats, documented by the crucial role of NO in limiting vessel constriction and in vessel wall remodeling (52).

On the other hand, insulin resistance causes selective impairment of the vasodilating and anti-atherogenic pathways by altered bioavailability or sensitivity to nitric oxide; in addition, it over activates the vasoconstrictor endothelin-1 (ET-1) production (53), and activates the renin-angiotensin-aldosterone system followed by upregulation of reactive oxygen species and downregulation of NO (54). Furthermore, hyperglycemia leads to increase in the formation of advanced glycation end products which in turn, activate protein kinase C, increasing expression of ET-1 (55) and reducing expression of eNOS (56).

Based on the aforementioned data and linking it to the present results, which indicated that exercise training and irisin injection induce a decrease in perineal fat, plasma insulin level and insulin resistance, associated with reduction in blood pressure and hyperglycemia together with

increase in nitrite level; marker of increased NO release, we speculate that the improved visceral obesity, glycemic control and insulin sensitivity with the increased NO, may be suggested mechanisms, mediating the blood pressure lowering effect of exercise and irisin.

In conclusion

This study showed that both moderate-intensity exercise intervention and irisin injection exhibit therapeutic, preventative, and protective effects against diabetic-induced hypertension, and visceral adiposity; however, the protective effect of irisin is more obvious, proved by the more lowering in FBG, insulin level, DBP, and perirenal fat and the higher nitrite levels compared to exercising group. These data suggest irisin as a therapeutic agent with a potential protective impact against diabetic complications. However, further researches to evaluate the irisin levels in serum and adipose tissue in T2DM-irisin group and their correlation to beiging process of adipose tissue are still warranted.

References:

1. **Kahn SE**: The relative contributions of insulin resistance and beta-cell dysfunction to the pathophysiology of type 2 diabetes. *Diabetologia*.; **46 (1)**: 3-19, 2003.
2. **Gastaldelli A**: Role of beta-cell dysfunction, ectopic fat accumulation and insulin resistance in the pathogenesis of type 2 diabetes mellitus. *Diabetes Res Clin Pract.;* **93**: S60-5, 2011.
3. **Sowers JR**: Insulin resistance and hypertension. *Am J Physiol*.; **286 (5)**: H1597-602, 2004.
4. **Dolinkova M, Dostalova I, Lacinova Z, Michalský D, Haluzikova D, Mraz M, and Haluzik M**: The endocrine profile of subcutaneous and visceral adipose tissue of obese patients. *Mol Cell Endocrinol*.; **291(1-2)**: 63-70, 2008
5. **Gheibi S, Kashfi K, and Ghasemi A**: A practical guide for induction of type-2 diabetes in rat: incorporating a high-fat diet and streptozotocin. *Biomed Pharmacother*.; **95**: 605-13, 2017a
6. **Yavari A, Najafipoor F, Aliasgarzadeh A, Niafar M and Mobasseri M**: Effect of aerobic exercise, resistance training or combined training on glycaemic control and cardiovascular risk factors in patients with type 2 diabetes. *Biol Sport*.; **29(2)**: 135-43, 2012.
7. **Warburton DE, Haykowsky MJ, Quinney HA, Blackmore D, Teo KK, Taylor DA and Humen DP**: Blood volume expansion and cardiorespiratory function: effects of training modality. *Med Sci Sports Exerc*.; **36(6)**: 991-1000, 2004
8. **Johnson NA, Sachinwalla T, Walton DW, Smith K, Armstrong A, Thompson MW, and George J:** Aerobic exercise training reduces hepatic and visceral lipids in obese individuals without weight loss. *Hepatology*.; **50(4)**: 1105-12, 2009.
9. **Stewart KJ:** Exercise training and the cardiovascular consequences of type 2 diabetes and hypertension: plausible

mechanisms for improving cardiovascular health. *JAMA*; ***288*(13)**: 1622-1631, 2002.

10. **Boström P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo J C, Rasbach KA, Boström EA, Choi JH, Long JZ and Kajimura S**: A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature*.; 481(7382): 463, 2012.
11. **Shoukry A, Shalaby SM, Bdeer SEA, Mahmoud AA, Mousa MM, and Khalifa A:** Circulating serum irisin levels in obesity and type 2 diabetes mellitus. *IUBMB life.*; ***68*(7)** 544-56, 2016.
12. **Kraemer RR, Shockett P, Webb ND, Shah U, and Castracane VD**: A transient elevated irisin blood concentration in response to prolonged, moderate aerobic exercise in young men and women. *Horm Metab Res*.; ***46*(02)**: 150-4, 2014.
13. **Zhang Y, Li R, Meng Y, Li S, Donelan W, Zhao Y, Qi L, Zhang M, Wang X, Cui T and Yang LJ**: Irisin stimulates browning of white adipocytes through mitogen-activated protein kinase p38 MAP kinase and ERK MAP kinase signaling. *Diabetes*.; **63(2)**: 514-25, 2014.
14. **Zhang W, Chang L, Zhang C, Zhang R, Li Z, Chai B, Li J, Chen E and Mulholland M**: Central and peripheral irisin differentially regulate blood pressure. *Cardiovasc Drugs Ther*.; **29(2)**: 121-7, 2015.
15. **Sesti G, Andreozzi F, Fiorentino TV, Mannino GC, Sciacqua A, Marini MA, and Perticone F**: High circulating irisin levels are associated with insulin resistance and vascular atherosclerosis in a cohort of nondiabetic adult subjects. *Acta Diabetol*.; **51(5)**: 705-13, 2014.
16. **Gheibi S, Bakhtiarzadeh F, Jeddi S, Farrokhfall K, Zardooz H, and Ghasemi A**: Nitrite increases glucose-stimulated insulin secretion and islet insulin content in obese type 2 diabetic male rats. *Nitric Oxide.*; **64**: 39-51, 2017b.
17. **Zhang M, Lv XY, Li J, Xu ZG, and Chen L**: The characterization of high-fat diet and multiple low-dose streptozotocin induced type 2 diabetes rat model. *Exp Diabetes Res*.; **2008**: 704045, 2009.
18. **Guerreiro LF, Pereira AA, Martins CN, Wally C, and Goncalves CAN**: Swimming physical training in rats: cardiovascular adaptation to exercise training protocols at different intensities. *J Exerc Physiol online*.; **18**: 1-11, 2015.
19. **Novelli ELB, Diniz YS, Galhardi CM, Ebaid GMX, Rodrigues HG, Mani F, Fernandes AAH, Cicogna AC, and Novelli-Filho JLVB**: Anthropometrical parameters and markers of obesity in rats. *Lab Anim*.; ***41*(1)**: 111-9, 2007.
20. **Wang Y, Song Y, Suo M, Jin X, and Tian G**: Telmisartan prevents high-fat diet-induced hypertension and decreases perirenal fat in rats. *JBR*.; **26(3)**: 219-25, 2012.
21. **Bonora E, Targher G, Alberiche M, Bonadonna RC, Saggiani F, Zenere MB, and Muggeo M**: Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of

insulin sensitivity: studies in subjects with various degrees of glucose tolerance and insulin sensitivity. *Diabetes Care*.; **23(1)**: 57-63, 2000.

22. **Cade WT**: Diabetes-Related Microvascular and Macrovascular Diseases in the Physical Therapy Setting. *Phys Ther*.; **88(11)**: 1322-35, 2008.
23. **Ibañez J, Izquierdo M, Argüelles I, Forga L, Larrión JL, García-Unciti M, Idoate F and Gorostiaga EM**: Twice-weekly progressive resistance training decreases abdominal fat and improves insulin sensitivity in older men with type 2 diabetes. *Diabetes Care*.; **28(3)**: 662-7, 2005.
24. **Johnson NA, Sachinwalla T, Walton DW, Smith K, Armstrong A, Thompson MW, and George J**: Aerobic exercise training reduces hepatic and visceral lipids in obese individuals without weight loss. *Hepatology*.; **50(4)**: 1105-12, 2009.
25. **Piao SJ, Kim SH, Suh YJ, Hong SB, Ahn SH, Seo DH, Park IS and Nam M**: Beneficial Effects of Aerobic Exercise Training Combined with Rosiglitazone on Glucose Metabolism in Otsuka Long Evans Tokushima Fatty Rats. *Diabetes Metab j*.; **41(6)**: 474-85, 2017.
26. **Mohammadi HR, Khoshnam MS, and Khoshnam E**: Effects of different modes of exercise training on body composition and risk factors for cardiovascular disease in middle-aged men. *Int J Prev Med.*; **9**:9, 2018.
27. **Pesta DH, Goncalves RL, Madiraju AK, Strasser B, and Sparks LM**: Resistance training to improve type 2 diabetes: working toward a prescription for the future. *Nutr Metab*.; **14(1)**: 24, 2017.
28. **Zhu G, Wang J, Song M, Zhou F, Fu D, Ruan G, Bai Y, Huang L, Pang R and Kang H**: Irisin Increased the Number and Improved the Function of Endothelial Progenitor Cells in Diabetes Mellitus Mice. *J Cardiovasc Pharmacol*.; 68(1): 67-73, 2016.
29. **Hou N, Liu Y, Han F, Wang D, Hou X, Hou S, and Sun X**: Irisin improves perivascular adipose tissue dysfunction via regulation of the heme oxygenase-1/adiponectin axis in diet-induced obese mice. *J Mol Cell Cardiol*.; **99**: 188-96, 2016.
30. **Mo L, Shen J, Liu Q, Zhang Y, Kuang J, Pu S, Cheng S, Zou M, Jiang W, Jiang C and Qu A**: Irisin is regulated by CAR in liver and is a mediator of hepatic glucose and lipid metabolism. *Mol Endocrinol*.; **30(5)**: 533-42, 2016.
31. **Xiong XQ, Chen D, Sun HJ, Ding L, Wang JJ, Chen Q, Li YH, Zhou YB, Han Y, Zhang F and Gao XY**: FNDC5 overexpression and irisin ameliorate glucose/lipid metabolic derangements and enhance lipolysis in obesity. *Biochim Biophys Acta.*; **1852(9)**: 1867-75, 2015.
32. **Saleh O, Majeed MJ, Oreaby GM**: Irisin impact as a medication that ameliorate and hinder the development of insulin resistance associated disorders without regular exercise (experimental study). *IOSR-JDMS*.; **13(9)**: 28-35, 2014.

33. **Lehnig AC, and Stanford KI**: Exercise-induced adaptations to white and brown adipose tissue. *J. Exp. Biol*.; **221(Suppl-1)**: jeb161570, 2018.
34. **Stanford KI, and Goodyear LJ**: Exercise regulation of adipose tissue. *Adipocyte*.; **5(2)**: 153-62, 2016.
35. **Yang G, Li C, Gong Y, Fang F, Tian H, Li J, and Cheng X**: Assessment of insulin resistance in subjects with normal glucose tolerance, hyperinsulinemia with normal blood glucose tolerance, impaired glucose tolerance, and newly diagnosed type 2 diabetes (prediabetes insulin resistance research). *J Diabetes Res.*; **2016**: 9270768, 2016
36. **Ghiasi R, Ghadiri Soufi F, Somi MH, Mohaddes G, Mirzaie Bavil F, Naderi R, Alipour MR**: Swim training improves HOMA-IR in type 2 diabetes induced by high fat diet and low dose of streptozotocin in male rats. *Adv Pharm Bull*.; **5(3)**: 379-84, 2015.
37. **Motahari-Tabari N, Shirvani MA, Shirzad-e-Ahoodashty M, Yousefi-Abdolmaleki E, and Teimourzadeh M**: The effect of 8 weeks aerobic exercise on insulin resistance in type 2 diabetes: a randomized clinical trial. *Glob J Health Sci*.; **7(1)**: 115-21, 2015.
38. **Colberg SR, Sigal RJ, Yardley JE, Riddell MC, Dunstan DW, Dempsey PC, Horton ES, Castorino K and Tate DF**: Physical activity/exercise and diabetes: a position statement of the American Diabetes Association. *Diabetes Care*.; **39**: 2065-79, 2016.
39. **Cartee GD**: Roles of TBC1D1 and TBC1D4 in insulin-and exercise-stimulated glucose transport of skeletal muscle. *Diabetologia*.; **58(1)**: 19-30, 2015.
40. **Bird SR, & Hawley JA**: Update on the effects of physical activity on insulin sensitivity in humans. *BMJ Open Sport Exerc Med*.; **2(1)**: e000143, 2017.
41. **Zhao J, Yang Z, He M, Wang Q, and Hu R**: Exercise preserves pancreatic β-cell mass and function in obese OLETF rats. *Ann Clin Endocrinol Metabol*.; **2**: 022-9, 2018.
42. **Ibrahim RH, and El-Malkey NF:** Role of irisin administration in modulating testicular function in adult obese albino rats. *Med. J. Cairo Univ.;* **86 (8)**: 4647-4655, 2018.
43. **Gizaw M, Anandakumar P, and Debela T**: A Review on the Role of Irisin in Insulin Resistance and Type 2 Diabetes Mellitus. *J pharmacopuncture*.; **20(4)**: 235-42, 2017.
44. **Chen JQ, Huang YY, Gusdon AM, and Qu S**: Irisin: a new molecular marker and target in metabolic disorder. *Lipids Health Dis*.; **14**: 2, 2015.
45. **Liu S, Du F, Li X, Wang M, Duan R, Zhang J, Wu Y and Zhang Q**: Effects and underlying mechanisms of irisin on the proliferation and apoptosis of pancreatic β cells. *PloS one*.; **12(4)**: e0175498, 2017.
46. **Perakakis N, Triantafyllou GA, Fernández-Real JM, Huh JY, Park KH, Seufert J, and Mantzoros CS**: Physiology and role of irisin in glucose

homeostasis. *Nat Rev Endocrinol*.; **13(6)**: 324-37, 2017.

47. Kadoglou NPE, Iliadis F, Angelopoulou N, Perrea D, Ampatzidis G, Liapis CD, and Alevizos M: The anti-inflammatory effects of exercise training in patients with type 2 diabetes mellitus. *Eur J Cardiovasc Prev Rehabil*.; **14(6)**: 837-43, 2007.
48. Sridhar B, Haleagrahara N, Bhat R, Kulur AB, Avabratha S, and Adhikary P: Increase in the heart rate variability with deep breathing in diabetic patients after 12-month exercise training. *Tohoku J Exp Med*.; **220(2)**: 107-13, 2010.
49. Lu Y, Li H, Shen SW, Shen ZH, Xu M, Yang CJ, Li F, Feng YB, Yun JT, Wang L and Qi HJ: Swimming exercise increases serum irisin level and reduces body fat mass in high-fat-diet fed Wistar rats. *Lipids Health Dis*; **15**: 93, 2016.
50. Fu J, Han Y, Wang J, Liu Y, Zheng S, Zhou L, Jose PA and Zeng C: Irisin lowers blood pressure by improvement of endothelial dysfunction via AMPK-Akt-eNOS-NO pathway in the spontaneously hypertensive rat. *Am Heart J*.; **5** : e003433, 2016.
51. Deng X, Huang W, Peng J, Zhu TT, Sun XL, Zhou XY, Yang H, Xiong JF, He HQ, Xu YH and He YZ: Irisin alleviates advanced glycation end products-induced inflammation and endothelial dysfunction via inhibiting ROS-NLRP3 inflammasome signaling. *Inflammation.;* **41(1)**, 260-75, 2018.
52. Ghimire K, Altmann HM, Straub AC, and Isenberg JS: Nitric oxide: what's new to NO? *Am J Physiol Cell Physiol*.; **312(3)**: C254-62, 2017.
53. Janus A, Szahidewicz-Krupska E, Mazur G, and Doroszko A: Insulin resistance and endothelial dysfunction constitute a common therapeutic target in cardiometabolic disorders. *Mediators Inflamm.*; **2016**: 3634948, 2016.
54. Muthaian R, Pakirisamy RM, Parasuraman S, Raveendran R: Hypertension influences the exponential progression of inflammation and oxidative stress in streptozotocin-induced diabetic kidney. *J Pharmacol Pharmacother*.; **7 (4)**: 159-64, 2016
55. Quehenberger P, Bierhaus A, Fasching P, Muellner C, Klevesath M, Hong M, Stier G, Sattler M, Schleicher E, Speiser W and Nawroth PP: Endothelin 1 transcription is controlled by nuclear factor-kappaB in AGE-stimulated cultured endothelial cells. *Diabetes*.; **49(9)**: 1561-70, 2000.
56. Xu B, Chibber R, Ruggiero D, Kohner E, Ritter J, and Ferro A: Impairment of vascular endothelial nitric oxide synthase activity by advanced glycation end products. *FASEB J.*; **17(10)**: 1289-91, 2003.
57. Lee Y, Kim JH, Hong Y, Lee SR, Chang KT, and Hong Y: Prophylactic effects of swimming exercise on autophagy-induced muscle atrophy in diabetic rats. *Lab Anim Res.*; **28(3):** 171-9, 2012.
58. Chen GQ, Mou CY, Yang YQ, Wang S, and Zhao ZW: Exercise training has beneficial anti-atrophy effects by inhibiting oxidative stress-induced MuRF1 upregulation in rats with diabetes. Life Sci.; **89(1-2):** 44-9, 2011.