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Effect of Chronic Exercise on Irisin Plasma Level and Browning of White Adipose Tissue in Rat Models with Obesity or Type 2 Diabetes Mellitus

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Keywords

- Irisin
- Browning
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- Chronic exercise

Abstract

Objective: To study the effect of swimming exercise training on plasma irisin level and browning of white adipose tissue in rats models with obesity or type 2 diabetes mellitus (T2DM). Methods: 60 male rats were divided into 4 groups, I) Control group, II) Exercise trained group, III) Obese group: includes 2 subgroups; subgroup A received high-fat diet and subgroup B received high-fat diet associated with daily exercise training and IV) Type 2 diabetic group: includes 2 subgroups; subgroup A and B. Both subgroups have T2DM. Subgroup B, in addition, was associated with daily exercise training after induction of T2DM. Plasma samples were analyzed for irisin, fasting insulin, fasting blood glucose and lipid profile. Levels of uncoupling protein-1 in subcutaneous abdominal adipose tissue homogenates (UCP-1 SAT) and uncoupling protein-1 in perinephric adipose tissue homogenates (UCP-1 PAT) were measured. Results: Subgroup IIIA had significantly higher irisin levels and significantly lower levels of UCP-1 SAT and UCP-1 PAT compared with group I. Subgroup IVA had significantly lower levels of irisin, UCP-1 SAT and UCP-1 PAT compared to controls. Chronic exercise significantly increased irisin, UCP-1 SAT, and UCP-1 PAT levels in group II, subgroup IIIB and subgroup IVB. Conclusion: Swimming exercise increases the plasma irisin level in normal, obese and type 2 diabetic rats inducing browning of white adipose tissue. Irisin is increased in obesity which may be attributed to a state of irisin resistance. T2DM is associated with lower plasma irisin levels.

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Introduction

Obesity is a main risk factor for the development of type 2 diabetes mellitus and cardiovascular disorders (1). Although obesity may induce insulin resistance and can predispose to high blood glucose level, T2DM also may develop in absence of obesity. Obesity has been found to contribute to approximately 55% of T2DM (2).

The adipose tissue pool is formed primarily of 2 different categories of lipids: white adipose tissue (WAT) and brown adipose tissue (BAT). White fat is the first place of energy reservoir and the secretion of adipocytokines that control body metabolism and insulin resistance (3). On the other hand, BAT has abundant mitochondria, also called power-houses of the cell, and uniquely expresses uncoupling protein-1 (UCP-1) also known as thermogenin. UCP-1 wastes the proton gradient of the inner membrane of mitochondria that is formed as an outcome of oxidative phosphorylation of food contents. This operation, which is called thermogenesis, produces heat in place of adenosine triphosphate (ATP) (4).

There are 2 developmentally distinct classes of brown adipocytes found in mammalians: the constitutive BAT (cBAT) that appears throughout embryogenesis; and the second type is the brite (brown in white) or "beige" adipose tissue that appears after birth in WAT or skeletal muscle, and has also been called recruitable BAT (rBAT) (5). The developmental source of cBAT is different from white adipocytes and rBAT, as its origin close to skeletal muscle. On the other hand, the synthesis of rBAT occurs through 2 pathways: either transdifferentiation of mature white to brown adipose cells (and vice versa) (6), or through initiation of differentiation of brown-adipogenic progenitors (7).

In several animal models, mainly rodents, a "browning process" has been reported which represents the appearance of brown-like adipocytes in white adipose tissue depots because of specific stimuli as chronic cold exposure or beta-adrenergic activation and other pharmacological and nutritional factors (8). The brown-like adipose cells which emerge in classical white adipose tissue depots have been known as "beige" adipocytes or recruitable brown adipocytes. These beige adipocytes express low levels of UCP1 compared with classic (constitutive) brown adipocytes however they are greatly inducible in case of suitable stimulation, which may, in these cells, switch on a strong plan of mitochondrial respiration and energy consumption like that of brown adipocytes (9).

Regular physical exercise has many benefits for fitness. Physical exercise increases muscular endurance and strength, consumes calories, and fights many diseases as obesity and type II diabetes. The effect of exercise is systemic and apparently can't be interpreted solely by consuming of calories in muscle (10).

A novel peptide was recently identified by Bostrom et al. (11) and has been called 'irisin' by the researchers. Irisin is an exercise-mediated myokine which regulates energy metabolism by increasing browning of WAT and so wastes chemical energy in the form of heat (11). This experimental work aimed to study the effect of swimming exercise training on plasma irisin level and browning of white adipose tissue in Sprague Dawely rats with obesity or T2DM.

Materials and Methods

➢ ***Chemicals***

Streptozotocin was obtained from Sigma Aldrich Chemical Co. (St. Louis, MO, USA). Fructose was produced by "El Nasr Pharmaceutical Company" and purchased from "El-Gomhouria Co. for Trading Chemicals, Medicines and Medical Appliances, Egypt."

➢ ***Animals***

60 Sprague- Dawley male rats weighing 100-120 gm. obtained from the animal house of Medical Experimental Research Center (MERC), Faculty of Medicine, Mansoura University. All experimental procedures were performed according to the guidelines of our local committee of animal research ethics. All animals were weighed twice per week and their food intake was monitored three times per week for 8 weeks. At the end of study period (8 weeks), rats were scarified and blood and adipose tissue samples were obtained.

➢ ***Experimental design***

The studied animals were classified into four groups as shown in figure-1.

1) Group I (control group, N= 10): Standard diet fed sedentary group, these animals were maintained on standard rat chow for 8 weeks. The standard rat diet consists of: 12.5% lipids, 63.2% carbohydrates, 24.3% protein (12).

2) Group II (exercise trained group, N= 10): Standard diet fed exercise trained group, these animals were maintained on standard rat chow for 8 weeks associated with daily exercise training.

3) Group III (Obese group, N= 20): This group was subdivided into 2 subgroups; subgroup A and B. Both subgroups were supplied daily with high fat diet for 8 weeks. Subgroup B, in addition, was associated with daily exercise training. The high fat diet consisted of 42% lipids, 36% carbohydrates, and 22% protein (kcal) (12).

4) Group IV (Type II diabetic group, N= 20): This group was subdivided into 2 subgroups; subgroup A and B. Both subgroups were supplied daily with 21% fructose solution for 4 weeks then after fasting overnight; they were injected intraperitoneal by single low dose of streptozotocin (30 mg/kg) to induce T2DM. Subgroup B, in addition, was associated with daily exercise training after induction of T2DM.

➢ ***Induction of type 2 diabetes mellitus***

Induction of type II diabetes was obtained by combination of high fructose feeding and a single small dose of streptozotocin that is considered as a simple and fast procedure to develop type II diabetes. Rats were supplied with 21% fructose solution with standard food for 4 weeks then after fasting overnight; they were injected intraperitoneal by single low dose of streptozotocin (30 mg/kg). Fructose was provided in water during the 1st 4 weeks then after streptozotocin injection animals were allowed free access to water. Fasting blood glucose levels were measured in blood samples obtained from rats' tails 3 days after STZ injection. Rats having fasting blood glucose level more than 200 mg/dl were considered diabetic (13). Type 2 diabetes lasted for 4 weeks in the diabetic group (group IV).

➢ ***Swimming exercise training***

Swimming exercise training was performed in normal (group II), obese (group IIIB) and type 2 diabetic (group IVB) rats. Rats were enabled to swim in a glass tank (80 cm diameter/ 100 cm height/ 40 cm water depth) filled with water kept

at 35 ± 1 C°. At the start of swimming exercise, rats were able to swim for 15 minutes, with additional 15 minutes daily till a swimming period of 1hour was obtained. Later on, a daily swimming period of 1hour, five times per week, was maintained for 8 weeks. At the end of each exercise session, animals were dried and maintained in a warm place (14).

➢ ***Blood sampling***

Prior to blood and tissue samples collection, animals were fasted overnight. In the morning, animals were weighed and scarified after anesthesia through intra-peritoneal (i.p.) injection of thiopental sodium in a dose of 120 mg /kg (15). The blood samples were collected by cardiac puncture and put in tubes with anticoagulant (heparin) for plasma samples collection. Plasma was separated by centrifugation at 1000 rpm for 15 minutes within 30 minutes of collection. The clean clear plasma was separated by Pasteur pipette, stored in dry sterile tubes, and then kept in a deep freeze at - 20°C until used for biochemical analysis.

➢ ***Preparation of adipose tissue homogenates***

After obtaining blood samples, adipose tissue samples were obtained from subcutaneous abdominal fat and perinephric fat. Adipose tissues removed from rats were stored at -80 C° for further analysis of UCP-1. Later on, one gram of frozen adipose tissue was rinsed with phosphate buffer saline to get rid of excess blood, then homogenized in 20 mL phosphate buffer saline and stored overnight at ≤ – 20 ^{0}C. Two freeze- thaw cycles were done to break the cell membrane then the homogenates were centrifuged for five minutes at 5000 x g. The supernatant was removed and stored at ≤ – 20 ^{0}C for subsequent analysis of UCP-1.

➢ ***Biochemical assays***

All plasma samples were analyzed for irisin, fasting insulin, fasting blood glucose (FBG) and lipid profile including total cholesterol (TC), triglycerides (TGs), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C). Plasma irisin level was assessed using commercial ELISA kits purchased from Biovender Company, Czech Republic. Plasma levels of glucose, TC, TGs, HDL-C were measured by spectrophotometer using colorimetric diagnostic kits supplied by Spinreact Company, Spain. The LDL-cholesterol was estimated according to the following formula, LDL-C = TC – HDL-C - TG/5 (16). Insulin was measured by rat insulin ELISA kit obtained from Shanghai Sun red biological technology company, China. Insulin resistance was calculated by mean of homeostatic model assessment of insulin resistance (HOMA-IR) (17). Subcutaneous abdominal and perinephric adipose tissue homogenates were analysed for the level of UCP-1 protein using commercial ELISA kits purchased from EIAab Company, China.

Statistical analysis

Data in the present work were expressed in the form of mean ± standard deviation (SD). Comparison of data was performed utilizing one way ANOVA test followed by Post Hoc Bonferroni test. Correlations between variables were performed by Pearson's

correlation coefficient. P value less than 0.05 was considered statistically significant.

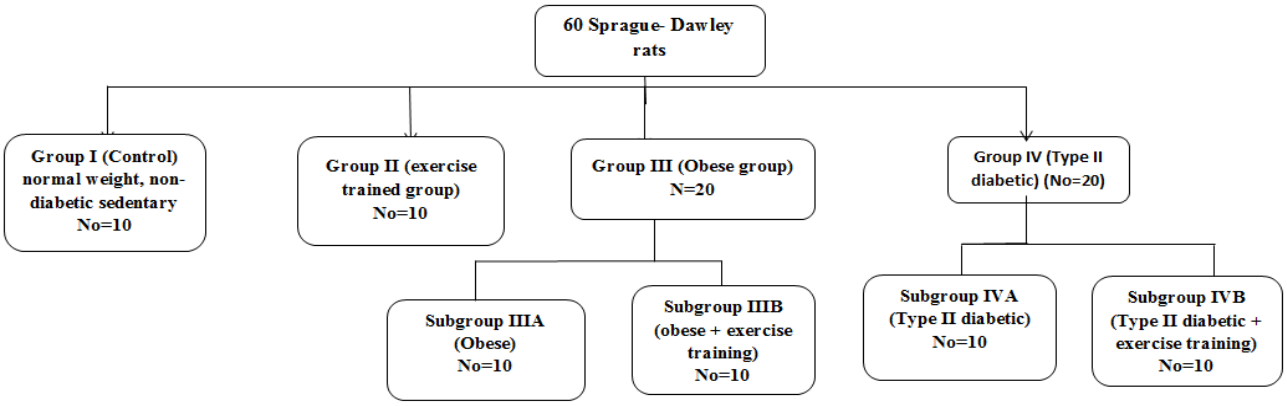

Figure (1): Distribution of the studied groups

Table (1) *Comparison between obese animals (group III) and controls (group I and II) regarding the laboratory data*

	Group I	Group II	Group III		P1	P2	P3	P4
			SG IIIA	SG IIIB				
Irisin (µg/ml)	**0.61±0.13**	**0.99±0.38**	**0.81±0.15**	**0.93** ± 0.05	**0.011***	**0.022***	**0.03***	**0.616**
UCP-1 SAT (ng/ml)	**1.42±0.88**	**2.74±0.93**	**0.75± 0.17**	**2.32±0.85**	**0.005***	**0.04***	**0.000***	**0.310**
UCP-1 PAT (ng/ml)	**1.6±0.85**	**2.63 ± 0.74**	**0.73±0.38**	**2.65±0.76**	**0.011***	**0.012***	**0.000***	**0.952**
FBG (mg/dl)	**95.6±8.53**	**81.1±5.72**	**109.5±17.16**	**96.10±4.45**	**0.013***	**0.04***	**0.038***	**0.000***
Fasting insulin (IU/mL)	**7.24±0.77**	**6.45±1.03**	**7.8±0.58**	**7.04 ± 0.67**	**0.07**	**0.08**	**0.015***	**0.151**
HOMA-IR	**1.7±0.15**	**1.29±0.15**	**2.11± 0.39**	**1.7±0.22**	**0.005***	**0.01***	**0.014***	**0.000***
Cholesterol (mg/dl)	**91.2±5.98**	**77.2±7.15**	**213.1± 13.76**	**196.4±13.28**	**0.001***	**0.000***	**0.013***	**0.000***
Triglycerides (mg/dl)	**66.3±9.96**	**52.7±4.57**	**166.1± 22.71**	**149.5±18.08**	**0.002***	**0.000***	**0.023***	**0.000***
HDL (mg/dl)	**40.4±3.92**	**49.3±5.08**	**29.8± 4.66**	**38.6** ± 4.53	**0.000***	**0.000***	**0.001***	**0.000***
LDL (mg/dl)	**37.1±2.65**	**17.35±5.83**	**150.08**± 18.33	**128.9** ± 12.82	**0.001***	**0.000***	**0.027***	**0.000***
FBW (gm)	**205.2**±20. 42	**132**±4.83	**373.6**±11.48	**296.2**±10.04	**0.000***	**0.000***	**0.000***	**0.000***

Data are presented as mean ±SD. G I (control), G II (control+ chronic exercise), SG IIIA (obese), SG IIIB (obese + chronic exercise). FBW: final body weight. Comparison of data was performed using one way ANOVA test. P1: compares G I and G II, P2: compares group I and SG IIIA, P3 compares SG IIIA and SG IIIB, P4 compares SG IIIB and G II * P value is significant (P<0.05).

Results

Table-1 shows plasma levels of irisin, FBG, fasting insulin, lipid profile and levels of UCP-1 SAT, UCP-1 PAT and final body weight (FBW) in group I (G I) (control), G II (control + chronic exercise), subgroup (SG) IIIA (obese) and SG IIIB (obese + chronic exercise). In exercise trained animals (G II), levels of irisin, UCP-1 SAT, UCP-1 PAT and HDL were significantly increased whereas FBG, HOMA-IR, TC, TGs, LDL-C and FBW levels were significantly decreased with non-significant change in fasting insulin levels when compared with group I. In

obese sedentary rats (SG IIIA), levels of irisin, FBG, HOMA-IR, TC, TGs, LDL-C and FBW were significantly increased whereas UCP-1 SAT, UCP-1 PAT and HDL levels were significantly decreased with non-significant change in fasting insulin levels compared with group I. In addition, obese rats experiencing chronic exercise (SG IIIB) showed a significant increase in irisin, UCP-1 SAT, UCP-1 PAT and HDL levels along with a significant decrease in FBG, fasting insulin, HOMA-IR, TG, TC, LDL and FBW levels compared with obese sedentary group (SG IIIA). Obese rats experiencing chronic exercise (SG IIIB) showed a significant increase in FBG, HOMA-IR, TC, TGs, LDL and FBW levels along with a significant decrease in HDL levels with non-significant change in irisin, , UCP-1 SAT, UCP-1 PAT and fasting insulin levels compared with exercise trained group (G II).

Table-2 shows plasma levels of irisin, FBG, fasting insulin, lipid profile and levels of UCP-1 SAT, UCP-1 PAT and FBW in G I (control), G II (control + chronic exercise), SG IVA (type 2 diabetic) and SG IVB (type 2 diabetic + chronic exercise). In type 2 diabetic rats (SG IVA), irisin, UCP-1 SAT, UCP-1 PAT and HDL levels were significantly decreased whereas FBG, fasting insulin, HOMA-IR, TC, TGs, LDL and FBW levels were significantly increased compared with group I. In addition, type 2 diabetic rats experiencing chronic exercise (SG IVB) showed a significant increase in irisin, UCP-1 SAT, UCP-1 PAT and HDL levels along with a significant decrease in FBG, fasting insulin, HOMA-IR, LDL and FBW levels with non-significant change in TC and TGs levels compared with type 2 diabetic sedentary group (SG IVA). Type 2 diabetic rats experiencing chronic exercise (SG IVB) showed a significant increase in FBG, fasting insulin, HOMA-IR, TC, TGs, LDL and FBW levels along with a significant decrease in irisin and UCP-1 SAT levels with non-significant change in UCP-1 PAT and HDL levels compared with exercise trained group (G II).

Table-3 showed significant positive correlations between plasma irisin levels and both UCP-1 SAT and UCP-1 PAT levels in all groups except SG IIIA. Significant negative correlations between plasma irisin levels and glucose levels were reported in G II and SG IVB. Significant negative correlation between plasma irisin level and fasting insulin level in SG IVB was reported. Significant negative correlations between plasma irisin levels and HOMA-IR levels were reported in SG IVB. Significant negative correlations between plasma irisin levels and TGs levels were reported in G II, SG IIIB and SG IVB. Significant negative correlation between plasma irisin level and FBW level in G II was reported.

Discussion

In the present study, obese rats of subgroup IIIA had significantly higher irisin levels and significantly lower levels of UCP-1 SAT and UCP-1 PAT compared with group I. These results are in agreement with Stengel et al. (18) and Pardo et al. (19) who reported that plasma irisin levels were significantly elevated in obese patients compared with normal weight subjects, and irisin also positively correlated with body weight, body mass index (BMI), and fat mass. However, Moreno-Navarrete et al. (20) and Choi et al. (21) reported that circulating irisin level was negatively

associated with BMI while the study by Sanchis-Gomar et al. (22) did not find a positive or negative correlation between circulating irisin levels and BMI.

Table-2 *Comparison between diabetic animals (group IV) and controls (groups I and II) regarding the laboratory data*

	Group I	Group II	Group IV		P1	P2	P3	P4
			SG IVA	SG IVB				
Irisin (µg/ml)	0.61±0.13	0.99 ± 0.38	0.27±0.08	0.48±0.22	0.011*	0.000*	0.01*	0.002*
UCP-1 SAT (ng/ml)	1.42±0.88	2.74 ± 0.93	0.74±0.29	1.5±0.8	0.005*	0.04*	0.016*	0.005*
UCP-1 PAT (ng/ml)	1.6±0.85	2.63 ± 0.74	0.85±0.34	2.27±0.98	0.011*	0.02*	0.000*	0.371
FBG (mg/dl)	95.6±8.53	81.1±5.72	267.4±14.58	255.4±4.77	0.013*	0.000*	0.031*	0.000*
Fasting insulin (IU/mL)	7.24±0.77	6.45±1.03	15.82 ± 2.24	13.06 ± 1.28	0.07	0.000*	0.004*	0.000*
HOMA-IR	1.7±0.15	1.29±0.15	10.45 ± 1.72	8.23 ± 0.79	0.005*	0.000*	0.003*	0.000*
Cholesterol (mg/dl)	91.2±5.98	77.2±7.15	193.6±20.91	180.5±6.77	0.001*	0.000*	0.087	0.000*
TGs (mg/dl)	66.3±9.96	52.7±4.57	111.9±23.61	97.6±9.62	0.002*	0.000*	0.102	0.000*
HDL (mg/dl)	40.4±3.92	49.3±5.08	34.7 ± 4.83	44.5±8.98	0.000*	0.01*	0.009*	0.163
LDL (mg/dl)	37.1±2.65	17.35±5.83	136.52 ±21.21	116.48±11.71	0.001	0.000*	0.028*	0.000*
FBW(gm)	205.2±20.42	132±4.83	274.3±7.36	243.8±6.71	0.000*	0.000*	0.000*	0.000*

Data are presented as mean ±SD. G I (control), G II (control+ chronic exercise), SG IVA (type 2 diabetic), SG IIIB (type 2 diabetic + chronic exercise). FBW: final body weight. Comparison of data was performed using one way ANOVA test. P1: compares G I and G II, P2: compares G I and SG IVA, P3 compares SG IVA and SG IVB, P4 compares SG IVB and G II * P value is significant (P<0.05).

Table-3 Correlations between plasma irisin levels and the laboratory data

		UCP-1 SAT	UCP-1 PAT	Insulin	Glucose	HOMA-IR	Chole s-terol	TGs	HDL	LDL	FBW
Group I	r	0.982	0.977	- 0.352	0.378	-0.424	0.005	-0.680	0.288	-0.367	-0.295
	p	0.000*	0.000*	0.318	0.281	0.223	0.988	0.031*	0.419	0.298	0.409
Group II	r	0.909	0.864	0.416	-0.642	0.252	-0.473	0.559	0.400	-0.440	-0.648
	p	0.000*	0.001*	0.232	0.045*	0.482	0.167	0.093	0.252	0.203	0.043*
Group III A	r	0.403	0.609	- 0.508	-0.623	-0.457	0.388	-0.551	-0.096	0.503	0.408
	p	0.294	0.061	0.133	0.054	0.184	0.268	0.099	0.791	0.138	0.242
Group III B	r	0.961	0.990	-0.149	-0.046	-0.106	0.058	-0.648	0.386	-0.032	0.271
	p	0.000*	0.000*	0.681	0.899	0.771	0.874	0.043*	0.270	0.931	0.499
Group IV A	r	0.744	0.938	-0.026	-0.045	-0.02	-0.100	0.061	-0.102	0.322	-0.137
	p	0.014*	0.000*	0.944	0.902	0.955	0.784	0.868	0.780	0.364	0.707
Group IV B	r	0.929	0.932	-0.680	-0.666	-0.642	0.522	-0.682	0.151	-0.396	-0.606
	p	0.000*	0.000*	0.031*	0.035*	0.045*	0.121	0.03*	0.677	0.257	0.063

r: Pearson correlation, **P:** significance (2-tailed).***** P value is significant (P<0.05).

Despite some controversy, it is generally believed that there is a positive correlation of circulating irisin with obesity, which is an apparent paradox with the proposed anti-obesity activity of irisin. A possible explanation which could reconcile the controversial data maybe that irisin acts as a physiological protective factor against obesity mediated by the browning of white adipose tissue and is therefore induced in compensation for increasing body mass. In cases of morbid obesity, physiological irisin can't preserve the balance between energy storage and consumption, and additional irisin is released from skeletal muscles, and even adipose tissues can produce irisin as compensation for dramatically increased fat storage (18, 19). Another explanation for high plasma irisin level and lower levels of UCP-1 SAT and UCP-1 PAT in obese rats is the probability of developing a form of "irisin-resistance" which may in part explain the high levels of this hormone in obese rats similar to leptin and insulin resistance (23).

As regard the relation between irisin and type II diabetes mellitus, our study showed that irisin plasma level was lower in type II diabetic rats of subgroup IVA in comparison with control group. This result agrees with Yan et al. (24) and Xiang et al. (25) who found that serum irisin levels were significantly decreased with new-onset T2DM. Many reports implicated PPAR-gamma co-activator-1α (PGC-1α) in pathogenesis of T2DM; it was observed that PGC-1α production and action were significantly decreased in the skeletal muscle of type 2 diabetic patients (26). In addition, Bostrom et al. (11) observed that exercise activates PGC-1α, which increases its downstream target fibronectintype III domain containing 5 (FNDC5), after that the C-terminus of FNDC5 protein is separated and the remaining 112 amino acid peptide is referred to as irisin (secretory portion of FNDC5 protein). Therefore, it is reasonable to propose that lower level of plasma irisin in type II diabetic rats observed in our work is due to impaired PGC-1α expression and activity in the skeletal muscle of diabetic rats.

Our study demonstrated that swimming exercise training significantly increased plasma irisin levels in group II, subgroup IIIB and subgroup IVB as exercise stimulates irisin release from skeletal muscles to induce beiging of WAT and thermogenesis (9), this result is in agreement with the work of Bostrom et al. (11) who observed that exercise increases PGC-1α production, which increases its downstream target FNDC5 protein (precursor of irisin).

In addition, our study showed that swimming exercise training in group II significantly increased the level of UCP-1 SAT and UCP-1 PAT as compared with group I. In addition, UCP-1 SAT and UCP-1 PAT levels in obese exercise trained group (subgroup IIIB) were significantly higher than untrained obese group (subgroup IIIA). Moreover, UCP-1 SAT and UCP-1 PAT levels in type II diabetic exercise trained group (subgroup IVB) was significantly higher than untrained type II diabetic group (subgroup IVA) indicating that exercise training can induce beiging of WAT depots. These results are in agreement with De Matteis et al. (27) who found that exercise training increased clusters of UCP-1 paucilocular and multilocular adipocytes in WAT (27). Also, exercise increases irisin secretion from skeletal muscles which regulates energy metabolism by

inducing browning of WAT and so wastes chemical energy in the form of heat. Therefore irisin can improve several metabolic disorders as obesity and T2DM (28).

Many hypotheses were proposed to elucidate the underlying molecular mechanisms that lead to beiging during exercise training. For instance, exercise is proved to enhance sympathetic innervation in subcutaneous WAT, the resultant increased sympathetic innervation can lead to beiging of subcutaneous WAT (29, 30). Cao et al. (31) reported that the beiging during exercise training occurs due to enhanced secretion of brain-derived neurotrophic factor from hypothalamus; however other researchers suggested that the several myokines secreted from skeletal muscles during exercise training may be responsible for beiging (32). These myokines include irisin (11), meteorinlike 1 (33), myostatin (34), and β aminoisobutyric acid (35). Although these hypotheses are intriguing, more investigations are necessary to completely clarify the mechanisms responsible for exercise-induced beiging of white adipose tissue.

In the present study, HOMA-IR in obese and type 2 diabetic rats (subgroup IIIA and IVA) was significantly higher than obese exercise trained and diabetic exercise trained rats respectively (subgroup IIIA and IVB) indicating that exercise improves insulin sensitivity. This result is in agreement with Chechi et al. (36) who demonstrated that transplantation of trained subcutaneous WAT in untrained recipient mice led to marked improvement in glucose tolerance and increased insulin sensitivity. This effect of exercise on insulin sensitivity may be attributed to the increase of brown-like adipocytes in subcutaneous WAT as a result of exercise training. Thus, the profound changes to WAT in response to exercise training may be part of the mechanism by which exercise improves whole-body metabolic health (36).

Regarding the correlations between plasma levels irisin and both UCP-1 SAT and UCP-1 PAT levels, the present study showed that irisin was positively correlated to UCP-1 SAT and UCP-1 PAT in all groups except subgroup IIIA. This indicates that irisin can induce browning of subcutaneous WAT and visceral WAT present in perinephric fat as irisin is released from skeletal muscles, stimulating the exercise-induced beiging of WAT via enhancing UCP 1 expression in white fat cells leading to augmented thermogenesis and consequently energy expenditure as described by Bostrom et al. (11). These beige or brown adipocytes in addition to brown fat itself, known to have a role in thermogenesis and energy expenditure as described by Wu et al. (9).

As regard the correlations between plasma irisin levels and plasma glucose levels, irisin showed significant negative correlations with plasma glucose levels in GII and SG IVB indicating that irisin can improve glucose tolerance and glucose uptake. This result is in agreement with Xin et al. (37) who demonstrated that irisin improved translocation of glucose transporter type 4 (GLUT4) in diabetic skeletal muscles (37). In addition, irisin seems to increase expression of GLUT4 in human mature adipocytes. However, whether this increase is also accompanied with improved GLUT4 translocation to the cell membranes (similar to that noticed in muscles) or with improved uptake of glucose in adipose tissues

remains unknown (38). Finally, irisin augmented lactate secretion from human mature adipocytes of subcutaneous adipose tissue, which indicates a stimulation glycolysis, possibly by decreasing oxidative respiration through uncoupling in the mitochondria (38).

Considering the correlations between plasma irisin levels and plasma triglycerides levels, significant negative correlations between plasma irisin levels and TGs levels were reported in G II, SG IIIB and SG IVB indicating that irisin can improve lipid profile. This finding is in agreement with Gao et al (39) who showed that irisin can control lipolysis. This was evidenced by a rise in glycerol content in the treated media, signifying that irisin may increase lipolysis in adipocytes. In addition, irisin augmented lipolysis-related genes expression in adipocytes. Also, irisin stimulated its own release in vitro by controlling FNDC5 expression (39). In addition, Xin et al. found that irisin increased the oxidation of fatty acids in myocytes and that knockdown of the adenosine monophosphate (AMP)-activated protein kinase (AMPK) decreased the effect of irisin on glucose uptake and fatty acid β-oxidation in myocytes. (37). Moreover, Zhang et al. showed that irisin is negatively correlated with intrahepatic triglyceride content in obese individuals (40).

From the current study we can conclude that swimming exercise increases the plasma irisin level in normal, obese and type 2 diabetic rats inducing browning of white adipose tissue. Irisin is increased in obesity which may be attributed to a state of irisin resistance. T2DM is associated with lower plasma irisin levels.

References:

1) Weyer C, Bogardus C, Mott DM, Pratley RE: The natural history of insulin secretory dysfunction and insulin resistance in the pathogenesis of type 2 diabetes mellitus. J Clin Invest 104: 787–94, **1999**.

2) Eberhardt MS, Ogden C, Engelgaum M & Cadwell, B: Prevalence of overweight and obesity among adults with diagnosed diabetes--United States, 1988-1994 and 1999-2002. *MMWR Morb Mortal Wkly Rep*, 53: (45), 1066-8, **2004**

3) Ronti T, Lupattelli G, Mannarino E: The endocrine function of adipose tissue: an update. Clin Endocrinol (Oxf) 64: 355–65, **2006**.

4) Cannon B and Nedergaard J: Metabolic consequences of the presence or absence of the thermogenic capacity of brown adipose tissue in mice (and probably in humans). Int J Obes (Lond) 34 (Suppl 1): S7–16, **2010.**

5) Ishibashi J & Seale P: Beige Can Be Slimming. *Science,* 328: (5982), 1113-1114, **2010**.

6) Cinti S : Between brown and white: Novel aspects of adipocyte differentiation. *Annals of Medicine,* 43: (2), 104-115, **2011**.

7) Lee YH, Petkova Anelia p, Mottillo Emilio p & Granneman James g: In Vivo Identification of Bipotential Adipocyte Progenitors Recruited by β3-Adrenoceptor Activation and High-Fat Feeding. *Cell metabolism,* 15: (4), 480-491, **2012.**

8) Granneman JG, Li P, Zhu Z, Lu. Y: Metabolic and cellular plasticity in white adipose tissue I: effects of beta3-adrenergic receptor activation. Am. J. Physiol. Endocrinol. Metab. 289: E608–E616, **2005**.

9) Wu J, Bostrom P, Sparks LM, Ye L, Choi JH,

Giang AH, Khandekar M, Virtanen KA, Nuutila P, Schaart G, Huang K, Tu H, van Marken Lichtenbelt WD, Hoeks J, Enerbäck S, Schrauwen P, Spiegelman BM: Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. Cell 150 (2): 366–76, **2012**.

10) Speakman JR & Selman C: Physical activity and resting metabolic rate. *Proceedings of the Nutrition Society*, 62: (03), 621-634, **2003**.

11) Bostrom P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC, Rasbach KA, Boström EA, Choi JH, Long JZ, Kajimura S, Zingaretti MC, Vind BF, Tu H, Cinti S,Højlund K, Gygi SP, Spiegelman BM: A PGC1-alpha-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. Nature 481 (7382): 463–8, **2012**.

12) Charbonneau A, Unsnon CG and Lavoie JM: High-fat diet-induced hepatic steatosis reduces glucagon receptor content in rat hepatocytes: potential interaction with acute exercise. J Physiol 579: 255-267, **2007.**

13) Wilson RD and Islam MS: Fructose-fed streptozotocin-injected rat: an alternative model for type 2 diabetes. Pharmacological Reports 64 (1): 129-139, **2012**.

14) Teixeira A, Trevizol F, Colpo G, Garcia S, Charao M, Pereira R, et al: Influence of chronic exercise on reserpine-induced oxidative stress in rats: Behavioral and antioxidant evaluations. Pharmacology Biochemistry and Behavior 88 (4): 465-472, **2008.**

15) Waynforth HB and Flecknell PA: Experimental and surgical technique in the rat. Academic Press Lomdon 2nd ed: 66-113**, 1998.**

16) Friedewald WT, Levy RI, Fredrickson DS: Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 18: 499-502, **1972.**

17) Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC: Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28 (7):412-9, **1985.**

18) Stengel A, Hofmann T, Goebel-Stengel M, Elbelt U, Kobelt P, Klapp BF: Circulating levels of irisin in patients with anorexia nervosa and different stages of obesity–correlation with body mass index. Peptides 39: 125–30, **2013.**

19) Pardo M, Crujeiras AB, Amil M, Aguera Z, Jimenez-Murcia S, Banos R: Association of irisin with fat mass, resting energy expenditure, and daily activity in conditions of extreme body mass index. Int J Endocrinol: 857270, **2014.**

20) Moreno-Navarrete JM, Ortega F, Serrano M, Guerra E, Pardo G, Tinahones F: Irisin is expressed and produced by human muscle and adipose tissue in association with obesity and insulin resistance. J Clin Endocrinol Metab. 98 (4): E769–78, **2013**.

21) Choi YK, Kim MK, Bae KH, Seo HA, Jeong JY, Lee WK: Serum irisin levels in new-onset type 2 diabetes. Diabetes Res Clin Pract. 100 (1): 96–101, **2013.**

22) Sanchis-Gomar F, Alis R, Pareja-Galeano H, Sola E, Victor VM, Rocha M: Circulating irisin

levels are not correlated with BMI, age, and other biological parameters in obese and diabetic patients. Endocrine 46 (3): 674–7, **2014.**

23) Crujeiras AB, Zulet MA, Lopez-Legarrea P, De La Iglesia R, Pardo M, Carreira M C, et al: Association between circulating irisin levels and the promotion of insulin resistance during the weight maintenance period after a dietary weight-lowering program in obese patients. Metabolism 63 (4): 520-531, **2014.**

24) Yan J, Feng Z, Liu J, Shen W, Wang Y, Wertz K: Enhanced autophagy plays a cardinal role in mitochondrial dysfunction in type 2 diabetic Goto-Kakizaki (GK) rats: ameliorating effects of (-)-epigallocatechin-3-gallate. J Nutr Biochem 23 (7): 716–24, **2012.**

25) Xiang L, Xiang G, Yue L, Zhang J, Zhao L: Circulating irisin levels are positively associated with endothelium-dependent vasodilation in newly diagnosed type 2 diabetic patients without clinical angiopathy. Atherosclerosis 235(2): 328–33, **2014**.

26) Soyal S, Krempler F, Oberkofler H, Patsch W: PGC-1α: a potent transcriptional cofactor involved in the pathogenesis of type 2 diabetes**.** Diabetologia 49: 1477–1488, **2006.**

27) De Matteis, R., Lucertini, F., Guescini, M., Polidori, E., Zeppa, S., Stocchi, V., et al: Exercise as a new physiological stimulus for brown adipose tissue activity. Nutrition, Metabolism and Cardiovascular Diseases 23 (6): 582-590, **2013**.

28) Chen, J.-Q., Huang, Y.-Y., Gusdon, A. M. & Qu, S. 2015. Irisin: a new molecular marker and target in metabolic disorder. *Lipids in Health and Disease*, 14: (1), 2.

29) Ranallo RF, Rhodes EC: Lipid metabolism during exercise. Sports Med 26: 29–42, **1998.**

30) Nedergaard J, Cannon B: The browning of white adipose tissue: some burning issues. Cell Metab 20: 396–407, **2014.**

31) Cao L, Choi EY, Liu X, et al: White to brown fat phenotypic switch induced by genetic and environmental activation of a hypothalamic-adipocyte axis. Cell Metab 14: 324–338, **2011.**

32) Pedersen BK, Febbraio MA: Muscles, exercise and obesity: skeletal muscle as a secretory organ. Nat Rev Endocrinol 8: 457–465, **2012.**

33) Rao RR, Long JZ, White JP, et al: Meteorin-like is a hormone that regulates immune-adipose interactions to increase beige fat thermogenesis. Cell 157: 1279–1291, **2014.**

34) Feldman BJ, Streeper RS, Farese RV Jr, Yamamoto KR: Myostatin modulates adipogenesis to generate adipocytes with favorable metabolic effects. Proc Natl Acad Sci 103: 15675–15680, **2006.**

35) Roberts LD, Boström P, O'Sullivan JF, et al: β-Aminoisobutyric acid induces browning of white fat and hepatic b-oxidation and is inversely correlated with cardiometabolic risk factors. Cell Metab 19: 96–108, **2014.**

36) Chechi K, Carpentier AC, Richard D: Understanding the brown adipocyte as a contributor to energy homeostasis. Trends Endocrinol Metab 24: 408–420, **2013.**

37) Xin C, Liu J, Zhang J, Zhu D, Wang H, Xiong L, Lee Y, Ye J, Lian K, Xu C, Zhang L, Wang Q, Liu Y, Tao L: Irisin improves fatty acid oxidation and glucose utilization in type 2 diabetes by regulating the AMPK signaling pathway. International Journal of Obesity 40, 443–451, **2016.**

38) Huh JY, Dincer F, Mesfum E, Mantzoros CS: Irisin stimulates muscle growth-related genes and regulates adipocyte differentiation and

metabolism in humans. Int. J. Obes. 38: 1538–1544, **2014.**

39) Gao S, Li F, Li H, Huang Y, Liu Y, Chen Y: Effects and Molecular Mechanism of GST-Irisin on Lipolysis and Autocrine Function in 3T3-L1 Adipocytes. PLoS One 11 (1), e0147480, **2016.**

40) Zhang HJ, Zhang XF, Ma ZM, Pan LL, Chen Z, Han HW, Han CK, Zhuang XJ, Lu Y, Li XJ, et al: Irisin is inversely associated with intrahepatic triglyceride contents in obese adults. J Hepatol 59: 557–562, **2013**.