



Irisin, an Exercise Stimulated Hormone as a Metabolic Regulator in Metabolic Syndrome in Obese Rats

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Abstract

Irisin plays an important role in energy metabolism and regulation of metabolic diseases as obesity and diabetes. This study aimed to evaluate the modulatory effect of physical exercise on the irisin as an exercise stimulated hormone and other biochemical parameters in obese rats suffering from metabolic syndrome (MS). Sixty male albino rats were randomly divided into 4 groups (15 in each): group I (control) healthy rats were fed on standard (St.) diet, group II (control+ex) in which rats were fed on St. diet and made exercise (ex.), group III: rats were fed on high carbohydrate high fat (HCHF) diet induced obesity in rats. Group IV (HCHF+ex.): rats also fed on HCHF diet and made exercise for 20 weeks. Serum glucose, insulin, lipid profile, irisin, Peroxisome proliferator-activated receptor -alpha (PPAR- α) and Interleukin 6 (IL-6) were measured. Histopathological examination of liver, pancreas and muscle was done. Our results revealed that glucose, lipid profile except HDL-C and IL-6 significantly increased, whereas a remarkable decrease in serum level of irisin and PPAR- α in HCHF group comparing to the control group. After swimming, all parameters were improved. Conclusion: swimming exercise has an efficient effect in improving all biochemical parameters of MS due to releasing of irisin which has regulatory mechanisms with respect to metabolic metabolism -associated health issues.

Keywords: Irisin , MS , PPAR- α , IL-6 , Physical exercise

1. Introduction

Metabolic syndrome (MS) is a worldwide health issue; it is well recognized in a number of metabolic disorders including obesity, insulin resistance, hypertension, and dyslipidemia. It is also known as syndrome X and Reaven syndrome [1]. High-carbohydrate and high-fat diet-induced oxidative stress have both been linked to the initiation of metabolic syndrome symptoms. In rats, high-carbohydrate and high-fat diets (HCHF) stimulate the symptoms of metabolic syndrome as dyslipidemia, impaired glucose tolerance, hypertension, elevated proinflammatory markers, lowered antioxidant defenses and increased fat deposition. [2].

Exercise is an essential mechanism for protection against cardiovascular diseases, and its positive effects on anti-inflammatory response have been recently revealed. A beneficial effect of exercise on type 2 diabetes, obesity and the metabolic syndrome is achieved via alterations in metabolism resulted from

modifications of quantity and / or activity of specific factors [3]. Since data regarding the effect of exercise training on key factors involved in metabolism are limited and controversial, the mechanisms by which exercise alters glucose and lipid metabolism are still unclear [4]. In the last decade novel peptides such as irisin, vaspin, visfatin, endothelin, as well as recently described adipons and salusins have been emerged as potential biomarkers involved in atherosclerotic and lipid disturbances [5].

Irisin has been initially characterized to protect against weight gain induced by diet, several studies have examined the correlation of circulating irisin with obesity in humans suggesting the role mediated by irisin in browning of white adipose tissue (WAT) increasing energy expenditure. Huerta-Delgado et al., [6] documented negative correlations between BMI and circulating irisin levels suggesting the protective function of the myokine irisin in obesity. Indeed, the effects of browning WAT could represent part of the

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longer-lasting benefits of physical exercise [7]. In addition, after acute exercise a mild increase in circulating levels of irisin by 3- fold enhanced expenditure of energy, reducing weight gain under high-fat diet, and enhancing diet stimulated insulin resistance [8]. Peroxisome proliferator-activated receptor -alpha (PPAR- α) is a ligand-activated transcriptional molecule. It was reported to have a significant role in different metabolic processes, as inflammation and atherogenesis [3, 9]. PPAR- α protein circulated eosinophilic expression is decreased in subjects with MS. This conclusion may help in the management of this worldwide health problem as it explains the accompanying obesity and endothelial dysfunction with MS [1]. By increasing genes responsible for intracellular fat acid transportation to peroxisome and mitochondria; PPAR- α is highly expressed in liver and can enhance oxidation of fatty acids. Recently several studies suggested that PPARs are involved in inflammatory control through regulating the duration, intensity and consequences of responses to inflammatory stimuli [3]. Long-term exercise could increase PPAR- α expression in liver improving hepatic metabolism which in turn benefits the metabolism of type 2 diabetes. In the same line, Zhang et al. [4] reported that in rats' liver increased PPAR- α expression is a contributory factor to the exercise-related improvements in whole-body metabolism. As a pleiotropic cytokine, IL-6 plays an important role in various metabolic processes as an autocrine and/or paracrine actions of adipocyte function. At present, accumulating evidences have demonstrated that IL-6 is closely linked to metabolic disorders such as MS and type 2 diabetes. Meanwhile, elevation of IL-6 has been documented in adipose tissues of patients with diabetes mellitus or obesity, particularly in those with features of MS. Eckel et al. [10] believed that the increase of IL-6 in MS appeared to act on several key factors, which contributed to insulin resistance, elevated glucose production in liver, together with inhibition of the insulin mediated glucose uptake in skeletal muscle [11]. During exercise, interleukin (IL)-6 is secreted from skeletal muscle (SkM) and it potentially affects hepatic metabolism. However, it is still unknown if SkM released IL-6 is implicated in the regulation of changes in carbohydrate and lipid metabolism in the liver induced by exercise training-counteraction in response to high-fat diet (HFD) feeding [12].

2. Aim of this study: we aimed to investigate the effect of the physical exercise on irisin, PPAR- α , IL-6 and other biochemical parameters and to evaluate the histopathological changes in obese rats suffering from MS.

3. Materials and Methods

Experimental study design: (Duration of experiment = 20 weeks) All experimental protocols were approved by Ethics Committee of the National Research Centre. Sixty male rats (4-6) weeks old, weighing 95 - 106 g, were obtained from Animal House of the National Research Centre. The rats were randomly divided into 4 experimental groups (15 rats/ group). The first group (control): 15 rats were fed with standard diet. Rats in the 2nd group (cont +ex) were fed on standard diet and made exercise according to Saleh et al. [13]. The third group (HCHF), rats were fed on HCHF diet whereas rats in the fourth group (HCHF+ex) were also fed on HCHF diet and made exercise for 20 weeks.

Diet-induced Metabolic Syndrome in Rats:

Feeding rats with high-carbohydrate high-fat (HCHF) diet (western style diet) was used in this study to induce a model more closely mimics to the changes observed in human metabolic syndrome (endothelial dysfunction, diabetes, obesity along with nonalcoholic fatty liver disease) according to Christopher et al. [14] and Fatma et al. [15]. Also, standard diet was prepared according to Reeves [16] and Fatma et al. [15] (Table 1).

Table (1) Component of western style (high fat diet) and standard diet (low fat diet):

product	HFF		LF	
	gm%	kcal%	gm%	kcal%
Protein	20	17	14.2	17.7
Carbohydrate	50	43	73.1	75.9
Fat	21	40	4.0	9.4
Total (kcal/gm)	4.68	100	3.85	100

High fat high fructose diet (HFF); Low fat diet (LF) as standard diet

Sampling:

Animals were fasted overnight, three ml of blood was aspirated under formalin anesthesia from the peripheral vein of the tail, then centrifuged for 15 min at 3000 rpm to obtain clear serum which stored at - 80°C till the day of the evaluation. Serum lipid profile and glucose level were measured immediately once every week to follow up the induction of obesity and diabetes.

Anthropometric measurements:

The weight of the rat in grams (gm) was measured by digital balance once every week.

Swimming Exercise:

Animals swam in a plastic tank (80 cm diameter/100 cm height/40 cm water depth) filled with water maintained at 35 ± 1 °C. Rats were habituated to the swimming exercise during the first week. Initially, rats swam for 15 min, with increments of additional 15 min

daily, until a swimming period of one hr was attained. Subsequently, a daily swimming period of one hr, 5 times/week, was maintained for 20 weeks. At the end of each exercise session, animals were dried and kept in a warm environment. Rats were sacrificed 48 hrs after last exercise session to minimize acute effects of exercise [17].

A-Biochemical parameters measured are:

Glucose (Fasting):

Glucose level in serum was measured according to the methods of Passing and Bablok, [18] by standard commercial colorimetric enzymatic assays (BioMerieux, Marcy l'Etoile, France; Roche Diagnostics, Basel, Switzerland).

Insulin :

Serum insulin level was estimated by enzyme linked immune sorbent assay according to Yallow and Bawman [19] using BioSoure INSEASIA Co. (Nivelles, Belgium) Kit.

Insulin resistance was calculated from the following equation: Insulin resistance=fasting glucose (mg/dl) ×fasting insulin (μIU/ml)/405, according to Mathews et al., [20].

Lipid profile:

Cholesterol, high density lipoprotein (HDL-cholesterol) and triglycerides (TG) levels in serum were measured according to the method of Kwang et al. [21], Lopez-Virella [22] and Cole et al., [23] respectively by standard commercial colorimetric enzymatic assays (BioMerieux, Marcy l'Etoile, France; Roche Diagnostics, Basel, Switzerland).

Determination of serum level of interleukin-6 (IL-6):

The levels of IL-6 (Pro-inflammatory adipokine) in the samples were determined using ELISA for rat according to Kimura and Kishimoto [24] using the manufacturers protocols (R&D systems).

Determination of serum level of irisin (Iris.):

The levels of irisin (myokine) in the samples were determined using Enzyme-linked immunosorbent assay (ELISA) for rat according to Samy et al. [25] using the manufacturers protocols (R&D systems).

Determination of serum level of peroxisome proliferator-activated receptor alpha (PPAR-α):

The levels of peroxisome proliferator-activated receptor alpha (PPAR-α) in the samples were determined using Enzyme-linked immunosorbent assay

(ELISA) for rat according to Lee et al. [26] using the manufacturers protocols (R&D systems).

Statistical Analysis

All analysis was done using the statistical package for the social science (SPSS) software version 9 on a personal computer. All numeric variables were expressed as a mean ± standard error (SE). The independent-sample T test was used to compare means. Pearson's correlation coefficient was obtained and a 'p' value < 0.01 was considered as statistically significant.

Histopathology:

Liver, pancreas and muscle specimens from the animals were dissected immediately after death, and fixed in 10% neutral-buffered formal saline for at least 72 hours. All the specimens were washed in tap water for half an hour and then dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin. Serial sections of 6 μm thick were cut and stained with Haematoxylin and eosin [27]. For histopathological investigation after one month of feeding. Images were captured and processed using Adobe Photoshop version 8.0.

4- Results:

-Table 2 showed a significant increase in weight upon feeding HCHF diet as comparing with normal control.

Table 3 recorded significant increase in glucose, insulin, IR, and cholesterol and TG in HCHF group as compared with the control; these results improved after exercise, whereas serum HDL show significant decrease upon feeding HCHF diet which modulated after exercise.

Our study showed that the level of IL-6 in a HCHF group was higher compared to the control group received standard diet (Table 4). These results showed a significant decrease in serum levels of irisin and PPAR- α in HCHF diet as compared to the control group whereas swimming exercise improves its level in both St. Diet and HCHF groups (Table 4).

The results revealed that, a strong positive correlation between irisin and PPAR-α in rats fed HCHF diet and made exercise (Fig 1) whereas a strong negative correlation between irisin and glucose as well as insulin suggesting that irisin may be involved in compensatory mechanisms for metabolic regulation (Fig 2,3).

Table (2) Mean weight of rats in different groups

	Control n=20	Cont + ex n=20	HCHF n=15	HCHF+ ex n=15	P-value
Base line weight (gm)	97.1 ± 1.6 ^a	97.7 ± 3.7 ^a	89.5 ± 6.1 ^a	101 ± 11.0 ^a	<0.001
Weight Gain (gm)	145.8 ± 7.0 ^b	128 ± 8.3 ^b	274 ± 9.6 ^c	223 ± 9.2 ^d	<0.001
% of weight gain	49.26	31.9	206	120.79	<0.001

Values are expressed as mean ± standard error (SE). Dissimilar values (superscripts a, b, c, d) of each row are significantly different.

Table (3) Mean serum levels of glucose, insulin, and IR and lipid profile in different groups

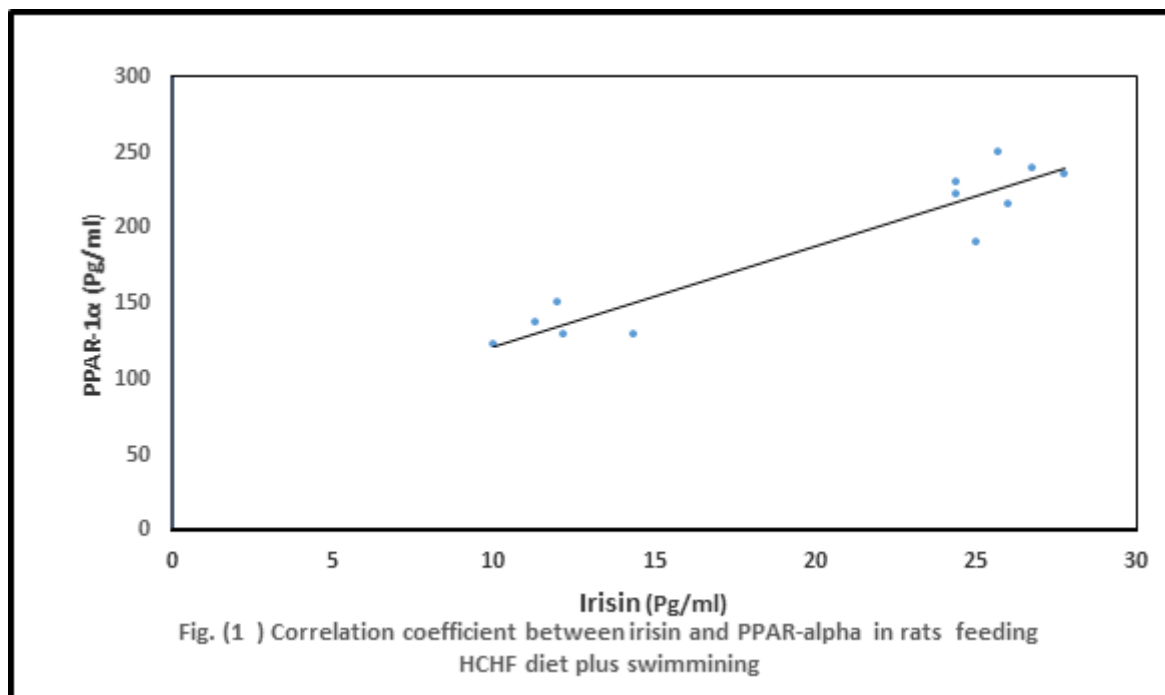
	Control n=20	Cont + ex n=20	HCHF n=15	HCHF+ ex n=15	P-value
Glucose (mg/dl)	87.15 ± 10.6 ^a	76 ± 10.1 ^a	163 ± 7.6 ^b	116 ± 4.7 ^c	<0.05
Insulin (Miu/l)	0.73 ± 0.02 ^a	0.41 ± 0.02 ^b	1.03 ± 0.08 ^c	.68 ± 0.04 ^a	<0.05
IR(mg/dl μIU/ml)	0.15 ± 0.01 ^a	0.05 ± 0.02 ^b	0.39 ± 0.03 ^c	0.19 ± 0.01 ^a	<0.05
Cholesterol (mg/dl)	57 ± 9.7 ^a	48.8 ± 8.1 ^a	180 ± 19.6 ^b	74.8 ± 14.1 ^c	<0.05
TG(mg/dl)	55.8 ± 17.6 ^a	31.8 ± 4.0 ^b	135 ± 15.3 ^c	125 ± 10.4 ^c	<0.05
HDL (mg/dl)	88.5 ± 0.7 ^a	104 ± 4.2 ^b	57.5 ± 3.5 ^c	95.5 ± 3.3 ^b	<0.05

Values are expressed as mean ± standard error (SE). Dissimilar values (superscripts a, b, c) of each row are significantly different.

Table (4) Mean serum levels of IL-6, irisin and PPAR-α in different groups

	Control n=20	Cont + ex n=20	HCHF n=15	HCHF+ ex n=15	P-value
IL-6 (pg/ml)	72.0 ± 2.2 ^a	67.8 ± 4.5 ^a	90.6 ± 11.9 ^b	80.4 ± 5.7 ^c	<0.055
Iris. (pg/ml)	22.4 ± 1.4 ^a	25 ± 1.2 ^a	17.4 ± 2.0 ^b	25.7 ± 3.7 ^a	<0.05
PPAR-α (pg/ml)	267 ± 66 ^a	378 ± 14 ^b	135 ± 12 ^c	219 ± 24 ^d	<0.05

Values are expressed as mean ± standard error (SE). Dissimilar values (superscripts a, b, c, d) of each row are significantly different.



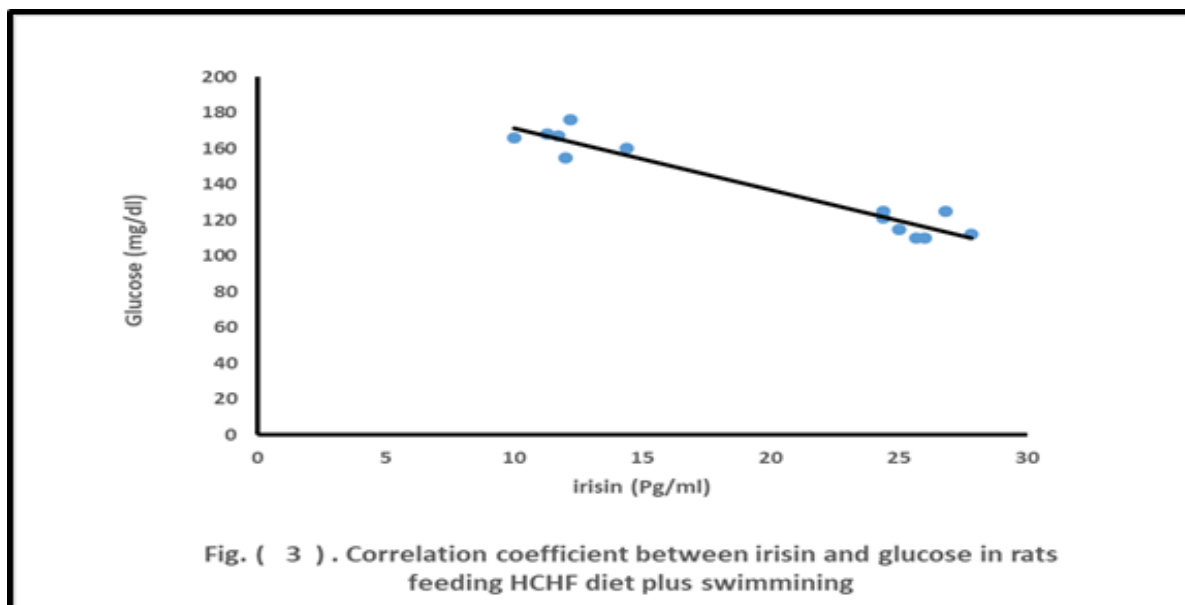
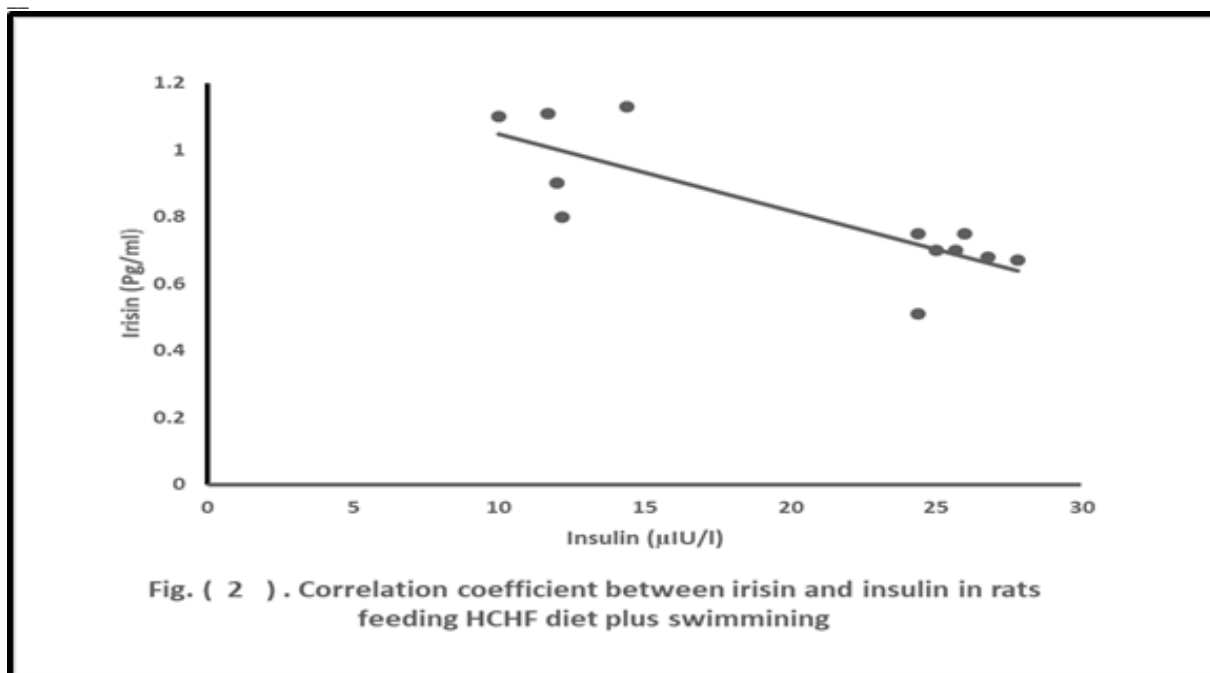


Table (5) Correlations between irisin, PPAR- α , insulin & glucose in HCHF+ex group

		irisin	PPAR	insulin(Miu/l)	Glucose
Irisin (pg/ml)	Pearson Correlation	1	.923**	-.815**	-.957**
	Sig. (2-tailed)		.001	.007	.000
	N	13	8	9	12
PPAR (pg/ml)	Pearson Correlation	.923**	1	-.922**	-.945**
	Sig. (2-tailed)	.001		.009	.000
	N	8	8	6	8
insulin(Miu/l)	Pearson Correlation	-.815**	-.922**	1	.671
	Sig. (2-tailed)	.007	.009		.068
	N	9	6	9	8
Glucose (mg/dl)	Pearson Correlation	-.957**	-.945**	.671	1
	Sig. (2-tailed)	.000	.000	.068	
	N	12	8	8	12

** . Correlation is significant at the 0.01 level (2-tailed).

5. Discussion

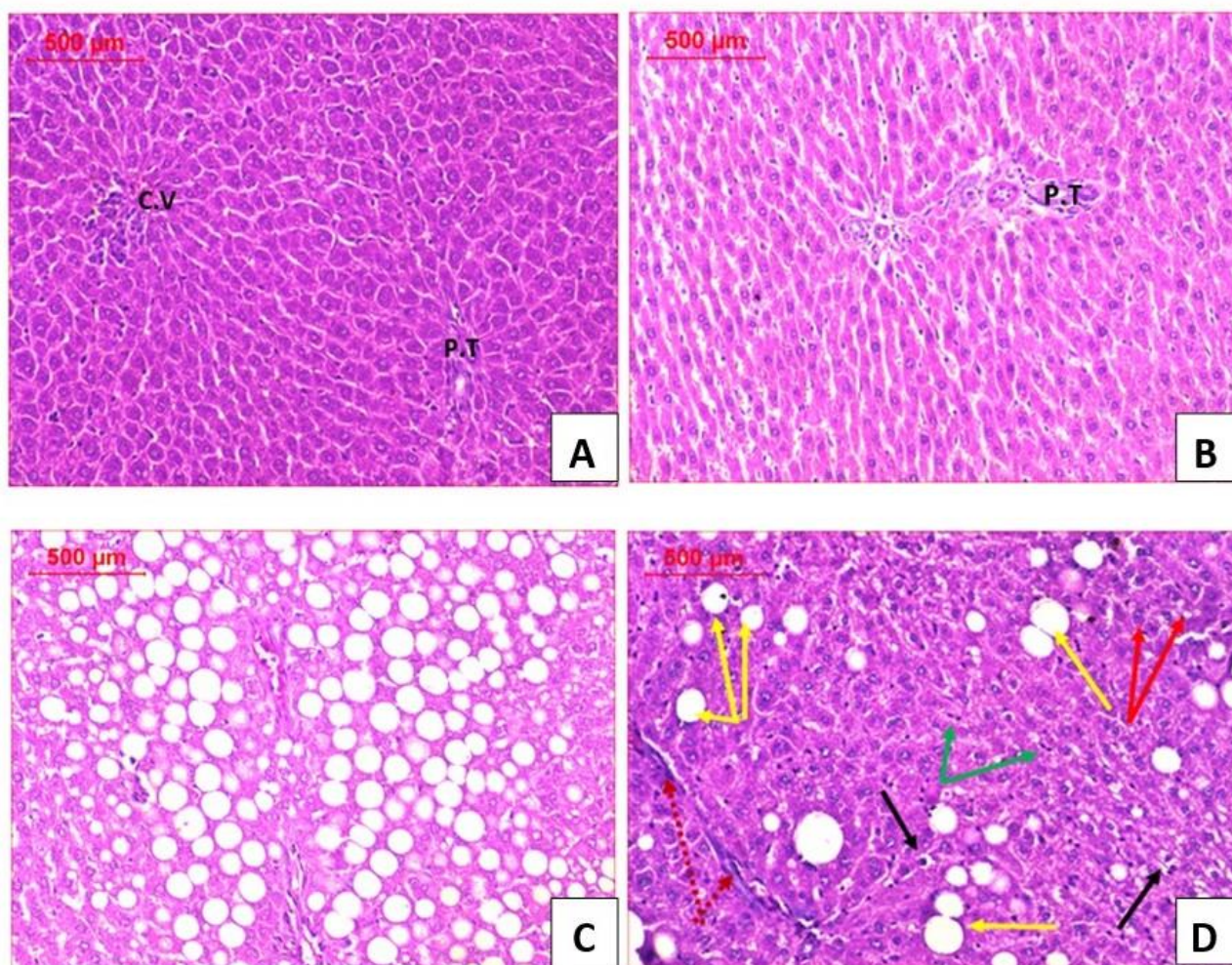
As MS affects the entire endocrine system, it causes physiological and biochemical abnormalities. Although the etiology of the disease is yet to be clarified, several studies implicate peptide hormones in its etiopathology [28]. Irisin, as a new hormone-like myokine, is discovered in the presence of exercise induced peroxisome proliferator-activated receptor gamma coactivator-1-alpha (PGC-1 α). This substance plays an important role in energy metabolism in each organ in the body and in the regulation of metabolic diseases such as obesity and diabetes [29].

The results showed that upon feeding HCHF diet, we noticed significant increase in weight (Table 2) whereas after exercise, there was a significant

improvement in the weight. This finding is in agreement with Chen et al. [29]. They noticed that once ge

nerated in muscle; this small peptide (irisin) will be secreted within the blood stream and translocated to adipose tissue or other organs or tissues for promoting energy metabolism regulation, browning of white adipocytes, improving insulin activity and decreasing insulin resistance, and optimizing body compositions. Concerning the lipid profile, we observed that TG and TC were higher in the HCHF group relative to control group (Table 3) in agreement with the results of More et al. [30]. This elevation may be due to the conversion of fructose to fructose 1-phosphate [31] and then converted by aldolase to trioses, enhancing the synthesis of fatty acids and triacylglycerols formation [32].

Histopathological Changes in Liver tissue:



Fig(4): A photomicrography of liver tissue for (A) Standard diet group, (B) Standard diet group + swim, (C) HCHF, (D) HCHF+swim., C.V; central vein, P.A; portal area, yellow arrows; fat cells, dashed red arrows; fibrotic strikes, black arrows; the hepatocytes are degenerated some with pyknotic nuclei, red arrows; others show necrotic nuclei, green arrows; activated kupffer cells (H&E200x).

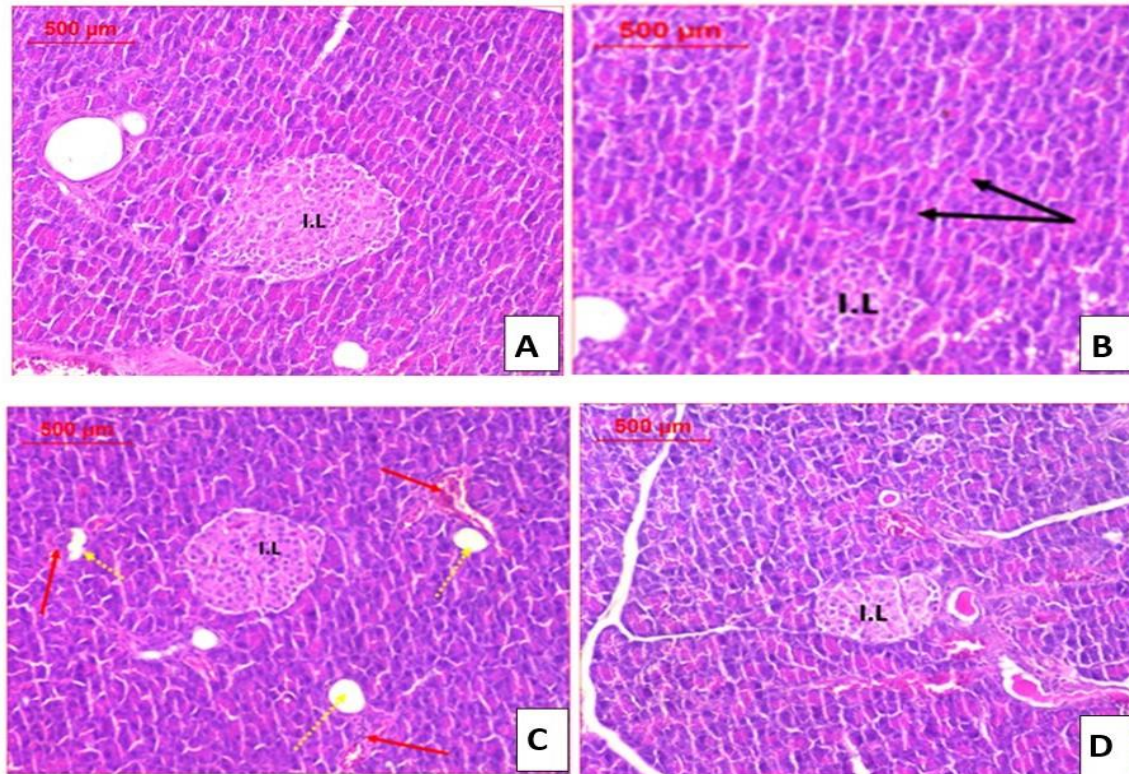
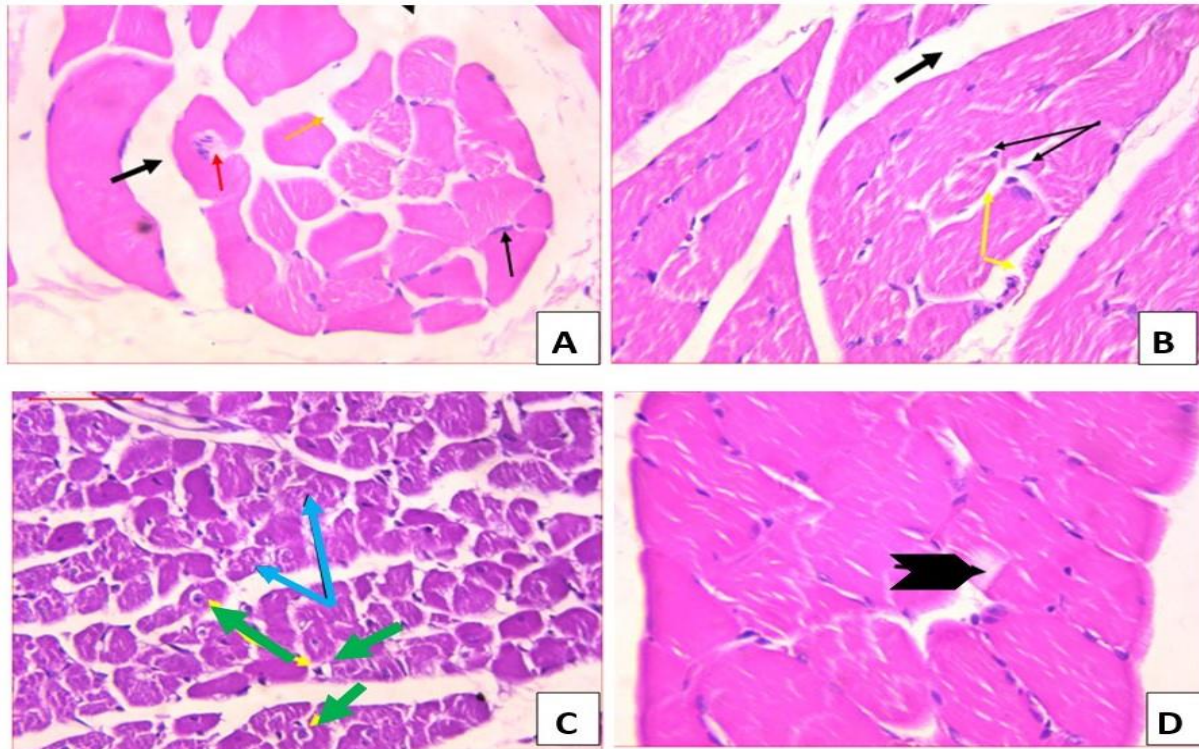
Histopathological Changes in Pancreas tissue:

Fig (5): A photomicrography of Pancreatic tissue for ; (A) Standard diet group, (B) Standard diet group + swim, (C) HCHF, (D) HCHF+swim, I.L; islets of Langerhans, black arrows ; exocrine pancreas in form of acini , dashed yellow arrows; areas of fatty deposition , red arrows ; congested vessels (H&E200x).

Histopathological Changes in Muscle tissue:

Fig(6): A photomicrography of skeletal muscle tissue for(A) Standard diet group, (B) Standard diet group + swim, (C) HCHF, (D) HCHF+swim, thin black arrows; muscle fibers with peripherally located nuclei, thin yellow arrow; endomysium, thick black arrow; perimysium, red arrows; blood capillaries, thick green arrows; foam cells, arrow heads; areas of hyalinization , blue arrows; signs of degeneration (H&E400x).

In our study, swimming exercise reduced TG level, TC and fat mass, in the HCHF group relative to the counterpart group (Table 3). These findings demonstrate that irisin may play a key role in the enhancement of fat metabolism along with exercise, indicating the accelerated consumption of energy by fat tissues is mainly due to irisin [33].

These findings suggest that irisin helps in the regulation of lipid metabolism, although the exact mechanisms of irisin action still unclear. Iglesia et al. [34] speculated that given irisin's putative effect as a 'metabolism-activator' and changes in irisin concentration might reflect a response to metabolic (or atherogenic) burdens. This might explain the positive correlation between irisin and atherogenic factors such lipid profile. In agreement with our results, Francisqueti et al. [35] reported that hyperadiposity produces adipokines, as well as proinflammatory cytokines such as TNF- α and IL-6 in subjects with MS suggesting a strong relationship between inflammation and obesity (Table 4).

Our study showed that the level of IL-6 in a HCHF group was higher compared to the control group received standard diet (Table 4). LDL cholesterol activates endothelial cells, macrophages lymphocytes, fibroblast and vascular smooth muscle to secrete IL-6 as soluble mediator of the inflammatory response that will diffuse from sub mucous lining into the vascular lumen, so that the high levels of IL-6 were detected in the blood of high-fat diet rats [36]. Upon swimming exercise we noticed that the concentration of interleukin (IL)-6 increases significantly during exercise in HCHF group compared to healthy rats (Table 4). The production of myokines as IL-6 is stimulated by physical exercise. As soluble factors released by skeletal muscle in response to muscle fiber contraction showing auto, para, and endocrine functions [37].

Peroxisome proliferator- α activated receptors (PPARs) have a crucial role in control of inflammation and metabolism [3]. Concerning the PPAR- α , our study showed significant decrease in its level in HCHF diet compared to the control group. Swimming exercise improves its level in both St. Diet and HCHF groups (Table 4). Lower serum triglyceride and high serum high-density lipoprotein cholesterol (HDL) levels were found to be associated with stimulation of PPAR- α receptors [3]. Moreover, a decrease in insulin resistance was found to be associated with PPAR- α receptors activation, maintaining its characteristic protective effects [38].

Hence, PPAR- α receptors are thought to have a key role in limiting atherosclerosis, owing to their effect in controlling inflammatory processes [39]. Exercise can regulate PPAR expression in macrophages, adipose tissue and skeletal muscle. These findings suggest that after acute exercise, PPAR- α is needed for

homeostasis of metabolic glucose, as it exerts an essential role on anti-inflammatory response mediated by exercise, and its absence probably enhances overexpression of proinflammatory cytokines [3].

In agreement to our data, Vosselman et al. [40] and Lu et al. [33] have reported elevation in serum irisin following chronic exercise in mice and humans. In contrast, Samy et al. [41] observed a fail to alter serum irisin levels with chronic swimming exercise which may be due to technical and physiological differences between studies in addition to species varieties. The different intensities of exercise and types (sprint, cycling or swimming; endurance or resistance) may have different influence on muscle disruption and metabolism, influencing concentrations of irisin. Timing of irisin measurement is another factor; several studies have tested irisin concentrations at different time points, so it is possible that irisin is elevated for a short period following exercise, after which it returns back to baseline [42].

Our results revealed that, a strong positive correlation between irisin and PPAR- α in rats fed HCHF diet and made exercise (Fig 1) whereas a strong negative correlation between irisin and glucose as well as insulin suggesting that irisin may be involved in compensatory mechanisms for metabolic regulation (Fig 2,3).

Histopathological examination of liver tissue revealed that the standard diet group showed normal hepatic architecture formed of multiple hepatic lobules (Fig 4.A), the same picture has been showed in (standard diet+swim) group (Fig 4.B). In agreement with Al Mamun et al. [43] the liver tissue of HCHF (Fig 4.C) showed hepatic architecture totally disrupted by foam cells (fatty accumulation) which much better improved in (HCHF+ swim) group (Fig 4.D).

The pancreatic tissue for standard diet group (Fig 5.A) and standard diet + swim group (Fig 5.B) showed the normal architecture formed of endocrine pancreas in form of islets of Langerhans and exocrine pancreas in form of acini are looking normal. The pancreatic tissue for high fat diet (Fig 5.C) showed exocrine acini are disrupted by areas of fatty deposition especially around the congested vessels, with minimal widen intercellular spaces, there are endocrinocytes showing signs of degeneration others with hypereosinophilic cytoplasm. The pancreatic tissue for HCHF+ swim showing the normal architecture formed of endocrine pancreas in form of islets of Langerhans with decreased number of islet cells (Fig 5.D).

The histopathological examination of skeletal muscle tissue transversely cut for standard diet group (Fig 6.A) showed muscle fibres with peripherally located nuclei, each one are separated from another by endomysium and a group of fibres make muscle fascicle which separated from each other by perimysium which include blood capillaries, the same

normal picture seen in standard diet + swim group (Fig 6.B). On the other hand the skeletal muscle tissue transversely cut for high fat diet (Fig 6.C) showed widely separated muscle fibres which interrupted with foam cells also some of fibres with signs of degeneration, HCHF+Swim showed separated muscle fibres with peripherally located nuclei with areas of hyalinization (Fig 6.D).

6. Conclusion

We concluded that irisin may be exploited as a novel target for the treatment of metabolic diseases or metabolism associated health issues, so as to accomplish prevention of metabolic disease or metabolism-associated health issues and improvement of life quality. All of these strategies will be particularly important for the people with limited physical activity or regular exercise because of disability.

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