IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 13, Issue 9 Ver. III (Sep. 2014), PP 28-35
www.iosrjournals.org

Irisin impact as a medication that ameliorate and hinder the development of insulin resistance associated disorders without regular exercise (experimental study)

Basil O. Saleh[1], Maysaa J. Majeed[1], Ghassan M. Oreaby[2]
[1] *Biochemistry of Department, College of Medicine, University of Baghdad, Iraq.*
Specialist of clinic immunology. Medical City, Baghdad, Iraq.

Abstract: *Slothful life style and lack of regular physical activity are the main causes of obesity, obesity–induced metabolic disorders. The aims of this study were to consolidation the harmony of serum Irisin with rats' fat development. Thinking over the innovation, can Irisin be therapeutic for metabolic disorders without regular exercise. This prospective experimental study was included 120 Western rats. The study had undergo two lines first was the correction of inducible obesity & diabetes; second was the prevention of mentioned inducible conditions, both lines was done by subjecting the rats to subcutaneous injection of prepared Irisin solution (100 ng/ml). Inducible obese type 2 diabetic (T2DM) rats had significantly higher serum Irisin, insulin level & fasting glucose (S.F.G.) in comparison with inducible obese rats & normal weight rats. Obese rats that were injected with 100 ng/ml of recombinant Irisin had highly significant lower weight mean and S.F.G. than obese rats control . Established diabetic rats with Irisin injection was significantly corrected, with their weight , serum glucose , and insulin after 26 days of irisin treatment. Findings showed that Irisin may act as reformation effect on new onset type 2 diabetics' rats; as well as it may have intensive defiance against fatness .*
Keywords -*Irisin, Obese Rats, Insulin Resistance ,Obesity Treatment Diabetic Rats, Rat Obesity Induction.*

I. Introduction

lifestyle factors such as poor quality diet, absence of physical activity are the most important factors that give rise to the development of overweight, obesity (defined by a body mass index (BMI) of greater than 25 kg/m2) & type 2 diabetes (T2DM) [1]. Animal models is a based tool for discussing the factors affecting humans owing to the highly analogy between the genomes of rodents and humans [2] ; that makes it possible for obesity and T2DM to be induced in experimental model and to investigate the causes and effects of obesity as well as supply a better understanding of the physiopathogeny of obesity, however it is necessary to choose the model that is best adapted to the characteristics to be studied. Whether they be environmental or genetic. Rates of type 2 diabetes have increased significantly since 1960 in parallel with obesity [3]. Obesity & diabetes were induced in normal adults' rats with age. Rats were fed with a variety of supermarket foods according to the international caloric value system obesity-induction model, by Hyper caloric diets (high fat diet). This is the simplest that closely gives the reality of obesity in human, by adding carbohydrates and fats, most of them vary between 3.7 and 5.4 kcal/g, developing insulin resistance ranged to level of diabetes mellitus [1] .

Two years ago, scientists discovered a new hormone called irisin which is released after moderate endurance aerobic activity (exercise). It has the ability to help maintain healthy body weight, that novel hormone with 112 amino acids has been identified by Wu et al. [4]. It is 100% conserved between mice, rat and human at the amino acid level. Spiegelman et al. [5] foresaw the effect of irisin as a 'messenger' declaring that, "There has been a feeling in the field that exercise 'communicate with the various tissues in the body. But the question that has been brought up is how?" At the time of irisin's initial discovery, Spiegelman et al. [5] suggested that it was the first step in explaining the biological mechanisms that translate physical exercise into beneficial changes throughout the body both in healthy people and in preventing or treating disease.

Irisin is capable of reprogramming the body's fat cells to burnt energy. Animal models have shown that irisin levels increase stimuli via regular aerobic exercise, but not during short-term bursts of anaerobic muscle activity; chronic exercise switches on genes that convert white fat into "good" brown fat. This helps people maintain a healthy BMI, avoid obesity, and conditions such as type 2 diabetes. These benefited effects of irisin gave the motivation to employ irisin supplements as therapeutics in drug development for the improvement insulin sensitivity without exercise.

II. Headings

II.1Experimental Study design: Sixty of them were put on regular standard diet (the pellet) and considered as control rats group and were also used for prevention studies, the other sixty remaining rats were subdivided into two groups. forty rats were used to induce obesity and 20 were used for diabetes mellitus induction. Sixty rats at

the age of 1.5 to 2 month- old were put on 200 g/day of regular standard diet (pellet) for ninety six days till the age of 4.5 to 5 months old. Forty of these rats were used as control group, of which sixteen were used for the determination of basal serum Irisin level. Forty rats at the age of 1.5 to 2 month old (as mentioned above) were fed with 200 g/ day of high fat diet to induce obesity, which consisted of 60% kcal fat according to Research Diets Inc. The obesity was approached and considered as obese rats group. Eight rats of this group were subjected to subcutaneous injection of prepared Irisin solution for 26 day, the second eight obese rats without Irisin injection therapy were considered as the control group Both groups were feed with same calories of high fat diet during this period. Twenty rats at age of 1.5-2.0 month-old were feed with 200 g/day of high carbohydrate and fat diet over the period of 4.5-5.5 month old age to induce and approach diabetes mellitus (T2DM), Seventeen of them were considered as diabetic rats group and their serum Irisin, insulin and glucose were measured at 4.5-5.0 months old, (approximately 4 months were needed for T2DM induction).Obese and Diabetic rats were subjected to Irisin subcutaneous solution injection protocol and was involved between one day and another injection of 150 µl of prepared Irisin (100 ng/ml). Changes in the weight and studied biochemical parameters were measured at both ages in groups.

Ten rats of normal weight were selected from the sixty normal weight control rats group, at age of 4.5 to 5.0 months. These ten rats had undergone grow-out system by depending on 200 g/day high fat diet for 26 days instead of pellet; the same system depends on protocol which was allocated for obesity induction. But at the time of obesity induction, five of them were subcutaneously injected with prepared Irisin, and were named as obese rats with Irisin injection treatment while the other five studied rats were named as obese rats without Irisin injection treatment, they were consider as control group for the first injectable obese rats.

Again ten studied rats were named as obese T2DM rat after developing insulin resistance, after treatment with Irisin injection but it is important to note that treatment was continued with the same high fat and CHO diet system.

П.2 Blood sampling: Two- three ml of blood was aspirated from the peripheral vein of the tail or by heart veinpunture, after the drawing process, blood was allowed to clot in vancover tubes, it was then centrifuged for 15 min at 3000 rpm to obtain clear serum which was stored at -20°C till the day of evaluation.

П.2 Anthropometric and biochemical measurements: The weight of the rat was measured by special scales, the weight was calculated in grams. Biochemical investigations involved serum fasting Irisin, insulin, glucose and prepared recombinant Irisin, animal were fasted overnight, until the next morning (from 11 pm to 9.0 am). Rats weight and serum glucose were measured once every week, blood from the tail was obtained under formalin anesthetized.

П.3a. Irisin Fc fusion, recombinant: Irisin Fc fusion is a form of Irisin that is fused to the Fc domain of human IgG in testing Irisin's biological activity . This fusion protein in principle renders stability of the protein *invitro* and *in vivo* without significant loss of activity [6].

The lyophilized Irisin Fc-Fusion, recombinant protein obtained from PHENIX PHARMACEUTICALS, INC.(GERMANY), was reconstituted with distilled water to obtain Irisin solution of 100 ng/ml concentration which is three times greater than that of normal serum Irisin of normal weight rats.

П.3b Irisin (Huma, Mouse and Rat): ELISA kit protocol, cat.No.:EK-067-52; Range 0.066-1024 ng/ml.

П.3c Rat Insulin ELISA Kit instructions (catalog≠ 90010) COMPANY: CRYSTAL CHEM INC(USA).

III. Indentations and Equations

III.1Results: Table (1), shed light on the difference of serum irisin among the three studied rats groups, which were selected at the of age 1.5 to 2.0 months old, the table also presented the basal weight at that age and the changes in their weight, serum fasting glucose and insulin levels after obesity and diabetes mellitus were induced at the age of 4.5 to 5.0 months old. The mean values of weight (295.59±30.30 g) and serum Irisin (31.84±6.35 ng/ml) of obese T2DM rats (aged 4.5-5 months old) were significantly higher when compared with those of normal weight rats (143.38±27.36 g, 21.49±3.02 ng/ml respectively) and obese (279.88±16.48 g, 26.71±4.62 ng/ml) rats for both (P< 0.001), with regard to weight & serum Irisin, the mean value of obese rats was significantly higher when compared with that of normal weight rats (P=0.001).

The mean values of serum glucose and insulin of obese diabetic rats (185.29±14.41 mg/dl, 3.5±2.1 ng/ml, respectively) were significantly increased when compared with those of normal weigh rats (84.67±10.83 mg/dl, p=0.001, 1.15±1.0 ng/ml, p=0.001) and obese rats (111.2±11.8 mg/dl, p=0.04, 2.16±2.0 ng/ml, p=0.001).

In obese rats group, the mean values of both glucose and insulin were also significantly increased when compared with normal weight rats (p=0.034, p=0.001, respectively).

To evaluate potential predictors of the circulating irisin, Rank correlation analysis of serum Irisin, rat's weight, metabolic factors (fasting serum glucose and insulin) were performed. Irisin level decline was

associated with weight gain in rats, showed by a significant negative correlation (r = - 0.66, p=0.005). Studied metabolic factors involved in the study as fasting serum insulin and glucose show independent changes associated with serum Irisin in rats with normal weight, however serum insulin changed negatively while serum glucose changed positively with serum Irisin in this group, that may reflect general view, although both show insignificant association.

Serum Irisin increment associated with the development of insulin resistance in healthy obese rats which reflect that through a positive correlation between serum Irisin & serum insulin in obese rats (r=0.57, p= 0.022). Serum glucose & weight of obese rat may reflect a positive correlation with serum Irisin but insignificant line (p>0.05), revealed independent association. The study didn't detect any dependent correlation between serum Irisin & weight, serum insulin, and serum glucose in rats with diabetes indication.

The recombinant Irisin injection solution for obesity correction, obese rats at the age of 4.5–5 months old were divided into two groups, mean (± SD) of their weight & S.F.G. at 5.5 -6.0 months old are illustrated in Table (2).

Data of Table (2) revealed that obese rats that were injected with100 ng/ml of recombinant Irisin had highly significant lower weight mean (± SD; 207.8±18.1 ng/ml) and S.F.G. (95.63 ± 6.23 mg/dl) than obese rats control with ($p < 0.001$, $p< 0.009$ respectively). Type 2 diabetes correction via 100 ng/ml of recombinant Irisin injection, analysis of variation between and within groups, obese T2DM rat before treatment with Irisin injection, obese T2DM rat after treatment with Irisin injection and obese T2DM rat after 10 days of with injection treatment are explained by Table (3). Established diabetic rats with Irisin injection, was significantly corrected for their weight (286.5±11.3 g, p= 0.03), serum glucose (144.8±18.0 mg/dl, p=0.001) and insulin (2.0±1.1 ng/ml, p=0.001) after 26 days of irisin treatment when compared with those rats without injection (weight, S.F.G. & insulin). The significant effect of irisin continue in weight (207.81±18.7 gm P=0.001), serum glucose (86.6± 10.93 mg/dl, p=0.001) and insulin (1.0±0.6 ng/ml, p=0.04) even after 10 days of stopping irisin injection treatment, which may also reflect the long half-life of irisin that is not identified till now.

Obesity prevention through 100 ng/ml recombinant Irisin injection is shown in Table (4). Irisin therapy successfully prevent the induction of obesity in rats as confirmed by significant weight reduction in rat subjected to injection and on high fat diet (208 ± 8.5 g, p=0.001) in comparison with their control.

In addition, the irisin therapy may also reduce the risk of insulin resistance as revealed by significant decrease in insulin level (p=0.03) reflected by the decrease of serum glucose level, even if it didn't reach the significant value.

Again, Table (5) proved the Irisin therapy effect in preventing the occurrence of diabetes mellitus by reducing the risk of insulin resistance. In addition to the useful prevention of developing obesity, Irisin also prevents the development of diabetes mellitus as confirmed by significant lowering of serum glucose (108.4±11.1 mg/dl, p=0.02), insulin (0.61±0.23 ng/ml, p=0.03) and weight (199.0± 7.10 g, p = 0.03) in rat subjected to subcutaneous injection with Irisin and which are on carbohydrate and fat diet in comparison with those subjected only to high carbohydrate and fat diet (without recombinant Irisin injection treatment) (207.5±52.7 mg/ml, 1.9±1.1 ng/ml,, 278.3± 32.2 g) respectively.

III.2 Discussion: Table (1) showed for the first description of this study a significant increase in circulating Irisin in obese rats which point to the indication reported by Roca-Rivadaet al. [7] and Roberts et al.[8] that Irisin is an Adipokine and Moreno-Navarreteet al. [9] who discovered that Irisin is expressed and secreted in both animals and humans by adipose tissue. In fact, they demonstrated that Irisin is a new adipokine with an important autocrine and endocrine functions. Rats with either directly induced obesity (DIO) or genetic obesity (Zucker rats) showed a significant increase of FNDC5/irisin secretion in both subcutaneous tissue and visceral adipose tissue compared with their lean counterpart. They also showed that short term period of exercise training induced FNDC5 secretion by WAT and that obese animal had an increase secretion of this hormone, suggesting a type of resistance. Another interesting feature reported by these authors in another study is that FNDC5/Irisin has different pattern of secretion depending on the anatomical location of adipose tissue in which the WAT of obese animals has a predominant secretary function.

The indication of these authors that FNDC5/Irisin has a secretion profile similar to other adipokines like leptin[6,10,11] found that circulated Irisin is secreted by adipose tissue FNCD5 and is correlated with several of metabolic factors. Bostrom et al. [6]also reported that WAT of obese animals over-secreted this hormone which might suggest a type of resistance because of 72% of circulated FNDC5-irisn was attributed to muscle secretion, adipose tissue secreted FNDC5/irisin which might participate in the remaining 28% [12].The most likely plausible explanation for the increased in exercise hormone with increase in rats weight as suggested by Cypess et al. [13], is that FNDC5/irisin showed a secretion pattern similar to other well-known adipokines (e.g. leptin); thus, their results also suggested a positive feedback pattern in which adipose tissue might be sensitive to FNDC5/irisin circulating levels. Because adipose tissue is determined to preserve its energy saving function

to maintain energy balance, the elevated levels of circulating FNDC5/irisin correspond to decreased levels of Irisin secretion by adipose tissue of studied animals.

The key feature of T2DM is insulin resistance. The development of insulin resistance is associated with the accumulation of lipid within skeletal muscle as excess intramuscular lipid metabolites impair insulin signaling, interfering with removal of glucose from the circulation [14]. A modest increase in PGC-1α levels increases mitochondrial biogenesis resulting in improvement in both lipid metabolism, mainly beta-oxidation pathway in the mitochondrial matrix, and increases in insulin-stimulated phosphorylation of insulin signaling proteins and in GLUT4 protein receptor in insulin resistant muscle of obese Zucker rats in vivo[15]. Fatty acid translocase (FAT) is a key fatty acid transporter of mitochondrial membrane which correlates with intramuscular lipid accumulation in obesity and T2DM [16] . A large muscle specific increase in PGC-1α (mRNA increased by 600%) provoked diet induced insulin resistance which was attributed to large increase in fat, while the modest over expression of PGC-1α, by ≈ 25%, insulin sensitivity was attenuated [16,17].

Chronic exercise is accompanied by increased muscle expression of PGC-1α, whereas T2DM or sedentary life style are associated with reduced expression [18,19] .The original report by Bostrom et al. [6] and Kurdiovaet al. [11] elucidated that FNDC5-Irisin was induced by both PGC-1α over expression and physical activity in mice.

The present experimental study revealed that circulating Irisin levels were significantly positively correlated with serum insulin in both obese rats that may point that increment in Irisin level is associated with increment in insulin level in obese rats or may reflect another figure that insulin increment guided to increment. This is highly confirmed that Irisin as hormone may act as a protective hormone against insulin resistance as a protective studied has shown. Serum Irisin was negatively associated with weight, but in normal weight rat, the most possible cause is the rats' growth or sex hormones effects , especially the study found out the negative association between serum Irisin & age in non-significant level, but in different comparison, the study put aside the growth factor in rats control group. In the diabetic rats groups, there was no loss of any significant correlation between serum Irisin and other studied parameters, the most possible cause of these findings was that this group was newly diagnostic, they did not develop the diabetes complication neither did the obese rats with just slightly insulin resistance. Choi et al.[19],Bostrom et al. [20] and Sanchis-Gomar et al.[21], have been postulated that irisin could serve as an injectable treatment for metabolic disease which may lead to improvement such as obesity and type 2 diabetes mellitus. The present study results in Tables (2&3) confirmed the positive effect of injectable Irisin in treatment of obesity as observed by significant decline of rats' weight and normalization of their blood glucose. These findings lead to questionable announcement about this hormone , such as ' is it beneficial or does it have a role in the treatment of metabolic disorder that mimics the exercise effect in the improvement of obesity related disorders.

On one hand, the possible suggestion about the hormone's action is that Irisin increment helps to turn white fat into the more beneficial and metabolically active brown fat, which burns more calories [22]. Investigation of the subcutaneous fat tissue depots in muscle-specific PGC-1α-overexpressing mice revealed a surprising finding [6]. Specifically, the white fat cells displayed signatures of brown fat cells, a feature referred to as "browning" , unlike white adipose, which is simply stores fat, brown fat is specialized to uncoupled mitochondrial respiration, thereby allowing the generation of heat. Thus, brown fat serves an important thermogenic function [15]. In addition, Irisin activated oxygen consumption and thermogenesis in white fat cells in culture, further, injection of an adenoviral vector expressing irisin into mice resulted in browning of subcutaneous white fat and increased total energy expenditure [23, 24, 25].

Moreover, increasing PGC-1α expression improves metabolic parameters such as insulin sensitively and insulin signaling [26], irisin was reported to induce a program of brown fat- like development in white adipose cells and to oppose high fat diet induced obesity and insulin resistance in mice [6] . Another study suggest a mechanism for the improvement of insulin sensitivity and insulin resistance by depending on the investigation at the molecular level on how irisin induces browning; in particular, it has been analyzed by the up regulation of UCP1 expression. Gene arrays indicates that one possible mechanism might be increased expression of PPAR-α which is a member of the PPAR family of ligand–activated receptors, which have become relevant as therapeutic targets owing to their roles in lipid and glucose metabolism and vascular biology [12]. Binding of irisin to unknown receptor on the surface of adipocytes in WAT changes their genetic profile, in particular irisin induce the expression of PPAR- α which is thought to be an intermediate downstream effecter that increases the expression of UCP1. The browning of WAT is associated with augmented mitochondrial density, oxygen consumption and increase in energy expenditure profile leading to favorable effects on metabolism [12]. Moreover, pharmacological inhibition with a PPAR-α- selective antagonist limited the induction of the browning of white adipose tissue by FNDC5 which are at least partially mediated via PPAR-αZhang et al. [27] recently reported that the irisin–gene UCP1 was mediated via cascades activation of p38 mitogen–activated protein kinase (p38 MAPK) and extracellular related protein kinase (ERK), in this fashion, they play a central

role in mediating the irisin browning function. PGC-1α is regulated by activation of Camp/PKA/p38 MAPK signaling pathways.

The second line of the present experimental design study which holds the improvement role of Irisin as a metabolic disorder preventable therapy is shown in Tables (4 & 5). The first identification report in this field [6] is that muscle specific expression of PGC-1α induced a brown like adipose tissue gene program. Mice over expressing PGC-1α observed markedly increased levels of BAT associated with transcripts, involving UCP1 and cidea in subcutaneous animal fat layer [28]. These opinion may give rise to workers mind that arrested development and suppression of gene of WAT may occurred, but this need to be confirmed by further molecular studies. The authors also hypothesized that modest prevention of weight gain, improvement of glycemic control and increased oxygen consumption were mainly related to irisin stimulating UCP1 expression in subcutaneous adipocytes by enhancing basal metabolism via increased oxygen consumption. This may reflect a powerful idea in researchers mind that irisin prevention action may be through increased of energy expenditure, a question that provoke, about the ways of energy exhaustion. The other explanatory mechanism for relevant action of irisin in alleviation of metabolic disorder is by improvement of whole-body aerobic capacity and glucose metabolism, this is associated with consistent changes in the metabolism–linked genes such as GLUT1, PDK4, and PDHA1 in human culture primary myotubes. Bourlier et al. [29] recently added another possible mechanism about irisin action that FNDC5 is abundant in heart muscle. Notably, that heart derived natriuretic peptides activate white adipose thermogenic programs [23]. The results of Bostrom et al. [6] suggest the intriguing possibility that organs involved in high energy expending activity, such as skeletal and heart muscle, send signals to fuel storage depots [30].

IV. Tables

Table (1): Descriptive characteristics of the study rats' samples (mean ±SD).

parameter	rats groups	No.	mean	SD	comparison of significant	
					ANOVA (f-test)	LSD (f-test)
weight (g) (age 1.5-2 months)	normal weight rat	16	86.69	11.59	0.16 NS	
	obese rat	16	86.16	7.84		
	obese T2DM rat	17	80.53	10.28		
weight (g) (age 4.5-5 months)	normal weight rat (A)	16	143.38	27.36	0.001 HS	A Vs B 0.001 HS
	obese rat (B)	16	279.88	16.48		A Vs C 0.001 HS
	obese T2DM rat (C)	17	295.59	30.30		B Vs C 0.001 HS
s.irisin (ng/ml) (age 4.5-5 months)	normal weight rat (A)	16	21.49	3.02	0.001 HS	A Vs B 0.38 NS
	obese rat (B)	16	26.71	4.62		A Vs C 0.001 HS
	obese T2DM rat (C)	17	31.84	6.35		B Vs C 0.00 HS
s.insulin (ng/ml) (age 4.5-5 months)	normal weight rat (A)	16	1.15	1.0	0.001 HS	A Vs B 0.03 S
	obese rat (B)	16	2.16	2.0		A Vs C 0.001 HS
	obese T2DM rat (C)	17	3.5	2.1		B Vs C 0.04 S
S.F.G. (mg/dl) (age 4.5-5 months)	normal weight rat (A)	16	84.67	10.83	0.001 HS	AVs B 0.001 HS
	obese rat (B)	16	111.2	11.8		A Vs C 0.001 HS
	obese T2DM rat (C)	17	185.29	14.41		B Vs C 0.001 HS

Table (2): Mean distribution of weight, S.F.G. in obese rats group that were injected with irisin and their control (Obese rats group without irisin injection).

parameter	studied groups	No.	mean	SD	t-test P-value	t-test Sig.
weights (g) (age5.5-6 months)	obese rats without inj. (control group)	8	343.6	37.7	0.001	HS
	obese rats with inj.	8	207.8	18.1		
S.F.G. (mg/dl) (age5.5-6 months)	obese rats without inj. (control group)	8	175.2	47.5	0.009	HS
	obese rat with inj.	8	95.63	6.23		

Table (3): Grading of diabetes correction responding to 100 ng/ml of recombinant Irisin injection.

studied parameter	studied groups	No.	mean	SD	comparison of significant ANOVA (f-test)	comparison of significant LSD (f-test)
weights (gm) (age 4.5-6.5 months-old)	obese T2DM rat before treatment with Irisin injection (A)	17	295.6	9.9	0.03 HS	0.001 HS A Vs B
	obeseT2 DM rat after treatment with Irisin injection (B)	10	286.5	11.3		0.001 HS B Vs C
	obese T2DM rat after 10 days of stopping treatment with Irisin injection (C)	8	207.81	18.1		0.00 1 HS A Vs C
s.Irisin (ng/ml) (age 4.5-6.5 months-old)	obese T2DM rat before treatment with Irisin injection (A)	17	31.84	6.35	0.001 HS	0.04 S A Vs B
	obeseT2DM rat after treatment with Irisin injection (B)	10	36.9	1.2		0.03 S B Vs C
	obese T2DM rat after 10 days of stopping treatment with Irisin injection (C)	8	29.3	1.1		0.001 HS A Vs C
S. insulin (ng/ml) (age 4.5-6.5 months-old)	obese T2 DM rat before treatment with Irisin injection (A)	17	3.5	2.6	0.001 HS	0.034 S A Vs B
	obeseT2 DM rat after treatment with Irisin injection (B)	10	2.0	1.1		0.001 HS B Vs C
	obese T2DM rat after 10 days of stopping treatment with Irisin injection (C)	8	1.0	0.6		0.04 S A Vs C
S.F.G. (mg/dl) (age 4.5-6.5 months-old)	obese T2 DM rat before treatment with Irisin injection (A)	17	190.3	14.41	0.001 HS	0.001 HS A Vs B
	obeseT2 DM rat after treatment with Irisin injection (B)	10	144.0	18.0		0.001 HS B Vs C
	obese T2DM rat after 10 days of stopping treatment with Irisin injection (C)	8	86.0	10.93		0.001 HS A Vs C

Table (4): Prevention of obesity in response to Irisin treatment.

studied parameter	rat fed with high fat diet	No	mean	SD	t-test p-value	t-test Sig.
weight (g) age: 4.5 - 4.5 months-old	rat feed with standard pellet diet for 3 months	5	177.8	10.9	0.8	NS
	rat feed with standard pellet diet for 3 months	5	179.3	9.9		
weight (g) age:5.5 -6.5 months-old	rat with high fat diet for 3 months (control)	5	223.5	11.1	0.001	HS
	rat with high fat diet but with Irisin injection treatment	5	208	8.5		
S.F.G. (mg/dl) age: 4.5 -6.5 months-old	rat with high fat diet for 3 months (control)	5	115.9	13.4	0.09	NS
	rat with high fat diet but with Irisin injection treatment	5	100	6.3		
serum insulin (ng/ml) age: 4.5 -6.5 months-old	rat with high fat diet for 3 months (control)	5	1.9	1.1	0.03	S
	rat with high fat diet but with Irisin injection treatment	5	0.61	0.23		

Table (5): Prevention of diabetes mellitus via Irisin therapy.

studied parameter	rats groups that were feed with high fat and CHO diet	No.	mean	SD	t-test p-value
weight (g) age 4.5- 5.5 months-old	rats fed with standard pellet diet for 3 months	5	180.7	10.9	0.8 NS
	rats fed with standard pellet diet for 3 months	5	178.9	11.1	
weight (g) age 4.5 -6.0 months -old	rats fed with high fat & CHO diet (control)	4	278.3	32.2	0.02 S
	rats fed with high fat & CHO diet but with irisin injection treatment	5	199.00	7.10	
S.F.G. (mg/dl) age 4.5 -6.0 months -old	rats fed with high fat & CHO diet (control)	4	207.5	52.7	0.03 S
	rats fed with high fat & CHO diet but with irisin injection treatment	5	108.4	11.1	
serum insulin (ng/ml) age 4.5 -6.0 months -old	rats fed with high fat & CHO diet (control)	4	1.9	1.1	0.03 S
	rats fed with high fat & CHO diet but with irisin injection treatment	5	0.61	0.23	

V. Conclusion

The current study findings revealed that all kinds of good implications in fighting obesity, diabetes were improved even after ten days of stopping irisin subcutaneous injection. This can give a possible assumption that the hormone effect remained for a period after the cessation of treatment. However the prolonged follow-up and serial irisin measurements which are not performed here may be a way for approximate defining the half-life of this new peptide. The long life of irisin was also proposed by Handschin and Spiegelman [18] who found that irisin blood levels in rats was still increase by 65% after 12 hours of rest beyond three weeks jogging regimen. Indication for the half-life of blood irisin may be reflected by the results of the present study more clearly from others.

Acknowledgements

We would like to introduce many thanks to all staff of obesity Center, Al-Kandy Medical College, Baghdad university, Teaching Laboratories, Baghdad Teaching Hospital, Diabetes Center, Al-Yarmouk Hospital, Al-Najaf Al- staff Hospital, also many thanks to the Department of Immunology, Teaching Laboratories for their assistance in laboratory hormones measurements, and to Animal House, College of Medicine, University of Baghdad for helping in the handling and caring of the rats.

References

[1]. Carey,DanielG,:Quantifying Differences in the Fat Burning ʾZone and Aerobic Zone:Implication For Training .Journal of Strength &Conditiong Research .2009.23.2090-2095.
[2]. Bagnasco M, Dube, MG, Katz, A, Kalra, S, &Kalra, SP. Models of diet induced obesity (DIO) are commonly used in research of non-leptin deficient obesity. Characterizations such as insulin resistance 1. Leptin expression in hypothalamic PVN reverses dietary obesity and hyperinsulinemia but stimulates ghrelin. Obesity Res 2003;11: 1463-1470.
[3]. Bergland CH. Irisin: The "Exercise Hormone" has Powerful Health Benefits Irisin can help maintain healthy weight, improve cognition, and slow aging. 2014 by in The Athlete's Way
[4]. Wu J, Bostrom, P, Spark LM, Choi LH et al. Beige Adipocytes Are a Distinct Type of Thermogenic Fat cell in mouse and Human. 2012; S0092-8674(12): 00595-8.
[5]. PuigserverP,AdelmantG,WuZ,FanM,Xu J,Ó MalleyB ,SpigelmanBM:ActivationofPPAR gamma coactivitor-1 through transcription factor.Science, 1999.286(5443):1368 -71.
[6]. Bostrom P, Wu J, Jedrychowski MP, Korde A, Ye L et al. A PGC-1- alpha-dependent myokine that drives brown-fat-likedevelopment ofwhite fat and thermogenesis. Nature 2012; 481: 463–468.
[7]. Roca-Rivada A, Castelao C, SeninLL et al. "FNDC5/ irisin is not only a myokine but also an adipokine,". PLoS One2013; Vol. 8,Issue 4,e60563.
[8]. Roberts MD, Bayless, DS. Company JM. et al. "Elevated Skeletal muscle irisin precursor FNDC5mRNAin obese OLETF rats," Metabolism 2013; 62(8):1052–1056.
[9]. Moreno-Navarrete JM, Ortega F, Serrano M, Guerra E, Pardo G, Tinahones F, Ricart W, Fern´andez-Real JM. Irisin is expressed and produced by human muscle and adipose tissue in association with obesity and insulin resistance. J ClinEndocrinolMetab 2013; 98: E769–E778.
[10]. Roca-Rivada A, Alonso J, Al-Massadi O, Castelao C, Peinado JR et al. Secretome analysis of rat adipose tissues shows location-specific roles for each depot type. J Proteomics 2011; 74: 1068–1079.
[11]. Kurdiova T, Balaz M, Vician M, Maderova D, Vlcek M, Valkovic L, Srbecky M, Imrich R, Kyselovicova O, Belan V, Jelok I, Wolfrum C, Klimes I, Krssak M, Zemkova E, Gasperikova1, D, Ukropec J, Ukropcova B. Effects of obesity, diabetes and exercise on FNDC5 gene expression and irisin release in human skeletal muscle and adipose tissue: in vivo and in vitro studies. J Physiol 2014; 592(5):1091-1107.

[12]. Hiukka A, Maranghi M, Matikainen N, Taskinen MR. PPARalpha: an emerging therapeutic target in diabetic micro-vascular damage. Nat Rev Endocrinol 2010; 6:454–463. [PubMed]
[13]. Cypess AM, Lehman S, William G et al. Identification and of brown adipose tissue in adult human, New England J Med 2009; 360(15): 1509 -1517.
[14]. Cusi K, Maezono K, Osman A et al. Insulin resistance differentially affects the PI 3-kinase- and MAP kinase-mediated signaling in human muscle. J Clin Invest 2000; 105:311–320.
[15]. Benton CR, Holloway GP, Han X-X, Yoshida Y, Snook LA, Lally J, Glatz JFC, Luiken JJFP, Chabowski A, Bonen A. Increased levels of peroxisome proliferator-activated receptor gamma, co-activator 1-alpha (PGC-1α) improve lipid utilization, insulin signalling and glucose transport in skeletal muscle of lean and insulin-resistant obese Zucker rats. Diabetologia 2010; 53: 2008–2019.
[16]. Højlund K, Staehr P, Hansen BF et al. Increased phosphorylation of skeletal muscle glycogen syntheses at NH2 terminal sites during physiological hyper-insulinemia in type 2 diabetes. Diabetes 2003; 52:1393–1402.
[17]. Stump CS, Short KR, Bigelow ML, Schimke JM, Nair KS. Effect of insulin on human skeletal muscle mitochondrial ATP protein synthesis, and mRNA transcripts. ProcNatlAcadSci 2003;100:7996–8001.
[18]. Handschin C, Spiegelman BM. The roleof exercise and PGC-1-alpha in inflammation and chronic disease. Nature 2008; 454: 463-469.
[19]. Choi YK, Kim MK, Bae KH, Seo HA, Jeong JY, Lee WK, Kim JG, Lee IK, Park KG. Serum Irisin levels in new–onset type 2 diabetes. Diabetes Res ClinPract 2013; 100(1): 96-101.
[20]. Bostrom PA, Boston MA, Mantzoros C. Irisin in humans: recent advances and questions for future research. MetabclinExper 2014; 63:178-180.
[21]. Sanchis-Gomar F, Lippi G, Mayero G, Perez-Quilis C, Jose L. et al. Irisin: A new potential hormonal target for the treatment of obesity and type 2 diabetes. J Diabetes 2012: 4:196
[22]. Wu J,BostromP,Spark L M…etal.Beige Adipocytes are adistinct Type of Thermogenic Fat cell in mouse and Human.2012:12:595-8.
[23]. Kelly DP. A newly discovered messenger system between muscle and fat tissue may explain the systemic benefits of exercise. Science 2012; 336: 42-43.
[24]. Laaksonen DE, Lindstrom J, Lakka TA, Eriksson JG, Niskanen L, Wikstrom K et al. Medicine. Irisin, light my fire. Science 2012; 336: 42–43.
[25]. Liu JJ, Wong MD, Toy WC et al. Lower circulating irisin is associated with type 2 diabetes mellitus. (J Diabetes Complications 2013; 27(4):365–9.
[26]. Wenz T, Rossi SG, Rotundo RL, Spiegelman BM, Moraes CT. Increased muscle PGC-1 alpha expression protects from sarcopenia and metabolic disease during ageing. ProcNatlAcadSci USA 2009; 106: 20405–20410.
[27]. Zhang Y, Li R, Meng Y et al. Irisin stimulates browning of white adipocytes through mitogen-activated protein kinase, MAP kinase and ERK MAP kinase signaling. Diabetes . 2014;63(2):514-25
[28]. CastilloIJ. From white to brown fat through the PGC-1α-dependentmyokineirisin: implications for diabetes and obesity. Dis Model 2012; 5(3):293-295.
[29]. Bourlier VS, aint-Laurent C, Louche K, Badin PM, Thalamas C, de Glisezinnski I, Langin D, Sengenes C, Moro C. Enhanced glucose metabolism is preserved in cultured primary myotubes from obese donors in response to exercise training. J ClinEndocrininolmetab, 2013:3739-47.
[30]. Soyal S, Krempler F, Oberkofler H, Patsch W. "PGC-1-alpha: a potent transcriptional cofactor involved in the pathogenesis of type 2 diabetes". Diabetologia 2006; 49: 1477–1488.