RESEARCH ARTICLE

STUDY THE EFFECT OF EXOGENOUS AND ENDOGENOUS IRISIN ON OBESITY AND TYPE II DIABETES MELLITUS IN MALE ALBINO RATS

Hanan Mostafa Abdallah, Sahar Ahmed Elsawy, Abeer Abed Ahmed and Rizk Mahmoud Elkholy
Medical Physiology Department, Faculty of Medicine, Tanta University, Egypt.

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Abstract

Irisin is a myokine secreted from muscle cells and other organs and it has multiple endocrinal effects on other body systems. The exact relationship of irisin in diabetes and obesity is still not completely understood.

Aim of the work: Is to study the effect of exogenous and endogenous irisin on obesity and type II diabetes mellitus in male albino rats.

Methods: 90 male albino rats divided into 3 groups. Control group; 10 rats that fed with a regular diet. Obese group; 40 rats that fed with high fat diet for 8 weeks then subdivided into 4 equal groups 10 rats each. Control obese group; treated by intraperitoneal (ip) injection of 150 μl saline daily for 8 weeks. Irisin treated obese group; treated by ip injection of 150 μl of prepared irisin solution daily for 8 weeks. Exercise treated obese group; treated by moderate intensity swimming exercise for 30 min per day 5 days a week for 8 successive weeks. Irisin and exercise treated obese group; treated as before. Diabetic group; 40 rats that fed with high fat diet for 4 weeks then injected with a single ip streptozotocin (STZ) in a dose of 30 mg/kg body weight. After one week, diabetic group subdivided into 4 equal groups 10 rats each. Control diabetic group, irisin treated diabetic group, exercise treated diabetic group and irisin and exercise treated diabetic group; treated as before.

Results: Non treated obese and diabetic groups showed significant increase in body weight gain, body mass index, serum insulin level, fasting glucose level, (HOMA-IR), osteopontin level, triglycerides level and atherogenic index of plasma. While, showed significant decrease in serum nitrite/nitrate level, HDL cholesterol level, irisin level and (HOMA-S) when compared to the control group. In contrast, irisin treated obese and diabetic groups showed significant decrease in body weight gain, body mass index, serum insulin level, fasting glucose level, (HOMA-IR), osteopontin level, triglycerides level and atherogenic index of plasma. While it showed significant increase in serum nitrite/nitrate level, HDL cholesterol level, irisin level and (HOMA-S) when compared to the control obese and control diabetic groups respectively. Treated obese and diabetic groups by moderate intensity swimming exercise for 8 successive weeks showed; significant changes in all parameters as compared to control obese and control diabetic groups respectively and insignificant changes in all
parameters as compared to irisin treated groups. Combination of irisin and exercise treated obese and diabetic groups showed insignificant change compared to control group and a significant change compared to either irisin treated groups or exercise treated groups except in body weight gain and body mass index there is no significant difference as compared to control group. Our work also showed that; serum irisin level is negatively correlated with all studied parameters except; insulin sensitivity, serum HDL cholesterol level and serum nitrite/nitrate level is positively correlated. We can conclude that combination therapy of irisin and exercise produce better results as anti hyperglycemic, anti-hyperlipidemic and antioxidant.

Introduction:

It has become increasingly recognized that skeletal muscle cells secrete signaling cytokines peptides referred as myokines which act in an autocrine, paracrine, and endocrine fashion in response to skeletal muscle contraction (e.g., exercise) and contribute to the immediate and chronic benefits of exercise (Mehrabian et al; 2016).

Irisin is a newly discovered hormone that is related to energy homeostasis and obesity (Pedersen and Febbraio 2012). It has direct significant impact on fat tissues (Boström et al., 2012) and is an apotential indicator of myocardial infarction (Kuloglu et al., 2014). Irisin is secreted into the circulation by contracting skeletal muscle after cleavage from the membrane protein fibronectin type III domain containing protein 5 (FNDC-5) (Seo et al; 2014). This hormone increases energy consumption and eventually reduces weight by affecting white and brown fats (He et al; 2015).

Diabetes mellitus is the most common chronic disease characterized by hyperglycemia resulting from defects in insulin secretion and/or activity (Shaw et al; 2010).

Obesity is one of the most important factors in the development of diabetes through various mechanisms including increased circulating free fatty acids, secretion of cytokines by white adipose tissue which ultimately exacerbates insulin resistance and decreased adiponectin which is cytokine derived from white adipose tissue and it has been linked to insulin sensitizing activity and cardiovascular protective properties (Choi et al; 2013).

Regular physical activity is found to play a key role in reducing the risk of obesity by increasing energy expenditure (Yun et al., 2016).

Materials and Methods:

Drugs and chemicals were obtained from (Sigma Aldrich co.) and all kits used for measurement of parameters were obtained from (Bio-diagnostic and fine test co.)

The rats were acclimatized for two weeks then categorized into:

Group I (Control group 10 rats): Rats of this group were fed with a regular diet.

Group II (Obese group 40 rats): Rats of this group were fed with high fat. after 8 weeks this group was subdivided into 4 equal groups 10 rats each (da Rocha et al; 2016).

1. Control obese group: Rats were treated by intraperitoneal injection of 150 μl saline daily for 8 weeks.

2. Irisin treated obese group: Rats were treated by intraperitoneal injection of 150 μl of prepared irisin solution (100 ng/ml) daily for 8 weeks (Basil et al; 2014).

3. Exercise treated obese group: Rats were treated by moderate intensity swimming exercise. It was performed without a load in a barrel filled with water at 33–35 °C to a depth of 40–50 cm, which allowed free swimming (Li et al; 2012). The duration of the first swimming exercise was limited to 15 min then increased by 5 min daily up to 30 min. Rats in the exercise groups swam for 30 min a day, 5 days a week for 8 successive weeks (Gobatto et al., 2001)

4. Irisin and exercise treated obese group: Rats were treated by irisin in addition to exercise treatment as before.
Group III (Type II diabetic group 40 rats): Type II diabetes (T2DM) was induced by feeding rats with a high-fat diet (HFD) as obese group for 4 weeks, then rats injected with a single intraperitoneal (i.p) administration of streptozotocin (STZ) in a dose of 30 mg/kg body weight (Sabitha et al., 2011). One week after STZ injection, the rats with FBG levels above 200 mg/dL considered as diabetic. After that this group was subdivided into 4 equal groups 10 rats each:
1. Control diabetic group: Rats were treated by intraperitoneal injection of 150 μl saline daily for 8 weeks.
2. Irisin treated diabetic group: Rats were treated by irisin as before.
3. Exercise treated diabetic group: Rats were treated by moderate intensity swimming exercise as before.
4. Irisin and exercise treated diabetic group: Rats were treated by irisin in addition to exercise treatment as before.

At the end of the experimental period, the following parameters were measured from all animals: Body weight gain, body mass index (BMI). The animals fasted overnight then, all rats were anesthetized by i.p injection of pentobarbital and blood samples were obtained and centrifuged at 3000 revolution per minute (rpm) for 15 minutes and the separated sera were then stored in aliquots at -30 °C till be used. Samples were thawed at room temperature at the time of assay measurement for estimation of the following parameters:
1. Serum insulin level: measured by radioimmunoassay (Kao et al., 1994).
2. Fasting blood glucose (FBG): measured according to the method of (Tietz, 1986).
3. Homeostatic model assessment of insulin resistance (HOMA-IR); calculated using the equation described by (Matthews et al., 1985).
4. Homeostatic model assessment of insulin sensitivity (HOMA-S); calculated using the equation described by (Matthews et al., 1985).
5. Serum Irisin; measured by enzyme linked immunosorbent assay (ELISA) (Kohl and Ascoli 2017).
6. Serum Osteopontin: measured by ELISA (Kohl and Ascoli 2017).
7. Serum high density lipoprotein (HDL) cholesterol level; measured according to method of (Grove, 1979).
8. Serum triglycerides (TG) level; measured by glycerol phosphate dehydrogenase (GPO) enzymatic method (Mcgowan et al., 1983)
9. Atherogenic index; Atherogenic index of plasma (AIP); log (triglycerides TG / high density lipoprotein cholesterol HDL-C) (Dobiásová, 2004).
10. Nitrite/ Nitrate level: was measured by colorimetric procedure according to the method described by (Montgomery and Dymock, 1961).

Statistical Analysis:
The data were analyzed using Statistical Program for Social Science (SPSS) version 22.0. Quantitative data were expressed as mean ± standard deviation (SD). The following tests were done: Independent- samples t-test of significance when comparing between two means. Correlation: to measure how strong a relationship is between two variables. Probability (P-value) less than or equal 0.05 was considered significant.

Results:-
Non treated obese and diabetic groups showed significant increase in body weight gain, body mass index, serum insulin level, fasting glucose level, (HOMA-IR), osteopontin level, triglycerides level and atherogenic index of plasma. While, showed significant decrease in serum nitrite/ nitrate level, HDL cholesterol level, irisin level and (HOMA-S) when compared to the control group.

In contrast, treated obese and diabetic groups by irisin showed significant decrease in body weight gain, body mass index, serum insulin level, fasting glucose level,(HOMA-IR), osteopontin level, triglycerides level and atherogenic index of plasma. While, showed significant increase in serum nitrite/ nitrate level, HDL cholesterol level, irisin level and (HOMA-S) when compared to the control obese and control diabetic groups respectively.

Treated obese and diabetic groups by moderate intensity swimming exercise for 8 successive weeks showed; significant changes in all parameters as compared to control obese and control diabetic groups respectively and insignificant changes in all parameters as compared to irisin treated groups.

Treated obese and diabetic groups by combination of both irisin and exercise showed insignificant change compared to control group and a significant change compared to irisin treated groups and exercise treated groups. This effect may be due to the potentiation between the effect of irisin and the effect of exercise in all parameters except in body weight gain and body mass index.
Our work also showed that; serum irisin level is negatively correlated with all studied parameters except; insulin sensitivity, serum HDL cholesterol level and serum nitrite/nitrate level is positively correlated.

Table 1: The mean value and standard deviation of measured parameters in obese groups.

| Parameter                        | Control group | Control obese group | Irisin treated obese group | Exercise treated obese group | Irisin and exercise treated obese group |
|----------------------------------|---------------|---------------------|---------------------------|------------------------------|----------------------------------------|
| Body weight gain in (gm)         | 83.7 ±39.28   | 149.4 ±54.91        | 22.5 ±41.13               | 27.7 ±33.36                  | -18.1 ±53.17                           |
| Body mass index (BMI) (gm/cm²)   | 0.596 ±0.106  | 0.950 ±0.140        | 0.667 ±0.141              | 0.681 ±0.142                 | 0.579 ±0.141                           |
| Serum insulin level (μIU/ml)     | 12.2 ±1.7     | 18.6 ±2.0           | 13.9 ±1.2                 | 14.0 ±1.3                    | 12.4 ±1.9                              |
| Serum FBG (mg/dl)                | 88.4 ±8.1     | 148.7 ±10.1         | 106.4 ±14.5               | 110.3 ±12.3                  | 83.9 ±8.4                              |
| HOMA-IR                          | 2.65 ±0.28    | 6.84 ±0.86          | 3.77 ±0.38                | 3.79 ±0.30                   | 2.55 ±0.36                             |
| HOMA-S                           | 0.329 ±0.005  | 0.290 ±0.004        | 0.315 ±0.007              | 0.313 ±0.003                 | 0.331 ±0.008                           |
| Serum irisin level (ng/ml)       | 37.9 ±3.0     | 28.8 ±3.4           | 69.7 ±4.4                 | 72.9 ±4.6                    | 84.6 ±4.6                              |
| Serum osteopontin level (ng/ml)  | 36 ±3.4       | 76.5 ±4.4           | 63.7 ±5.9                 | 66.7 ±5.5                    | 38.1 ±3.8                              |
| Serum HDL cholesterol level(mg/dl)| 41.1 ±4.0   | 24.0 ±4.1           | 34.9 ±4.2                 | 36.4 ±4.1                    | 41.7 ±3.9                              |
| Serum triglycerides level(mg/dl) | 137.5 ±7.5   | 176.7 ±9.8          | 147.3 ±9.8                | 145.3 ±7.3                   | 134.3 ±7.7                             |
| Atherogenic index of plasma (AIP)| 0.525 ±0.049 | 0.871 ±0.080        | 0.662 ±0.040              | 0.602 ±0.042                 | 0.508 ±0.037                           |
| Nitrite/ Nitrate level (μmol/L)  | 15.9 ±1.921  | 9.3 ±1.23           | 11.5 ±1.56                | 11.1 ±1.32                   | 15.4 ±1.77                             |

a, b, c, d Denote statistical significance at p ≤ 0.05.
aVs control group.
bVs control obese group.
c Vs irisin treated obese group.
dVs exercise treated obese group.

Table 2: The mean value and standard deviation of measured parameters in diabetic groups.
| Parameter                          | group  | group          | diabetic group | treated diabetic group |
|-----------------------------------|--------|---------------|----------------|------------------------|
| Body weight gain in (gm)          | 83.7   | ±39.28        | 28.8<sup>a,b</sup> | 33.3<sup>a,b</sup> ±35.21 | -13.5<sup>a,b</sup> ±30.55 |
| Body mass index (BMI) (gm/cm<sup>2</sup>) | 0.596  | ±0.106        | 0.856<sup>a</sup> ±0.181 | 0.646<sup>b</sup> ±0.082 | 0.644<sup>b</sup> ±0.101 | 0.601<sup>b</sup> ±0.185 |
| Serum insulin level (μIU/ml)      | 12.2   | ±1.7          | 19.3<sup>a</sup> ±1.8 | 14.7<sup>a,b</sup> ±1.2 | 14.7<sup>a,b</sup> ±1.4 | 11.9<sup>a,b,c,d</sup> ±1.9 |
| Serum FBG (mg/dl)                | 88.4   | ±8.1          | 276.1<sup>a</sup> ±17.1 | 180.5<sup>a,b</sup> ±18.5 | 185.0<sup>a,b</sup> ±12.7 | 83.9<sup>a,b,c,d</sup> ±8.4 |
| HOMA-IR                          | 2.65   | ±0.28         | 13.1<sup>a</sup> ±1.45 | 6.55<sup>a,b</sup> ±0.49 | 6.74<sup>a,b</sup> ±0.96 | 2.49<sup>b,c,d</sup> ±0.53 |
| HOMA-S                           | 0.329  | ±0.005        | 0.268<sup>a</sup> ±0.003 | 0.291<sup>a,b</sup> ±0.002 | 0.291<sup>a,b</sup> ±0.005 | 0.333<sup>a,b,c,d</sup> ±0.010 |
| Serum irisin level (ng/ml)        | 37.9   | ±3.0          | 30.3<sup>a</sup> ±2.4 | 68.7<sup>a,b</sup> ±3.9 | 70.8<sup>a,b</sup> ±4.2 | 92.6<sup>a,b,c,d</sup> ±3.3 |
| Serum osteopontin level (ng/ml)   | 36     | ±3.4          | 79.2<sup>a</sup> ±4.7 | 65.5<sup>a,b</sup> ±4.8 | 65.5<sup>a,b</sup> ±4.2 | 37.3<sup>a,b,c,d</sup> ±3.3 |
| Serum HDL cholesterol level(mg/dl) | 41.1   | ±4.0          | 25.7<sup>a</sup> ±3.8 | 35.7<sup>a,b</sup> ±4.5 | 36.2<sup>a,b</sup> ±5.5 | 40.2<sup>a,b,c,d</sup> ±4.7 |
| Serum triglycerides level(mg/dl)  | 137.5  | ±7.5          | 170.9<sup>a</sup> ±9.4 | 145.7<sup>a,b</sup> ±9.4 | 145.7<sup>a,b</sup> ±7.3 | 133.4<sup>a,b,c,d</sup> ±6.8 |
| Atherogenic index of plasma (AIP) | 0.525  | ±0.049        | 0.825<sup>a</sup> ±0.074 | 0.612<sup>a,b</sup> ±0.052 | 0.607<sup>a,b</sup> ±0.063 | 0.522<sup>a,b,c,d</sup> ±0.061 |
| Nitrite/ Nitrate level (µmol/L)   | 15.9   | ±1.921        | 7.6<sup>a</sup> ±0.99 | 9.8<sup>a,b</sup> ±1.04 | 9.6<sup>a,b</sup> ±1.22 | 15.5<sup>a,b,c,d</sup> ±2.23 |

a, b, cand dDenote statistical significance at p ≤ 0.05
aVs control group.
bVs control diabetic group
cVs irisin treated diabetic group.
dVs exercise treated diabetic group.
Figure 1a: Body weight gain in (gm) of obese groups

Figure 1b: Body weight gain in (gm) of diabetic groups

Figure 2a: Body mass index (BMI) (gm/cm²) in obese groups

Figure 2b: Body mass index (BMI) (gm/cm²) in diabetic groups

Figure 3a: Serum insulin level (μIU/ml) in obese groups

Figure 3b: Serum insulin level (μIU/ml) in diabetic groups

Figure 4a: Serum fasting glucose level (FBG) (mg/dl) in obese groups

Figure 4b: Serum fasting glucose level (FBG) (mg/dl) in diabetic groups
Figure 5a: Homeostatic model assessment of insulin resistance (HOMA-IR) in obese groups

Figure 5b: Homeostatic model assessment of insulin resistance (HOMA-IR) in diabetic groups

Figure 6a: Homeostatic model assessment of insulin sensitivity (HOMA-S) in obese groups

Figure 6b: Homeostatic model assessment of insulin sensitivity (HOMA-S) in diabetic groups

Figure 7a: Serum irisin level (ng/ml) in obese groups

Figure 7b: Serum irisin level (ng/ml) in diabetic groups

Figure 8a: Serum osteopontin level (ng/ml) in obese groups

Figure 8b: Serum osteopontin level (ng/ml) in diabetic groups
Figure 9a: Serum HDL cholesterol level (mg/dl) in obese groups

Figure 9b: Serum HDL cholesterol level (mg/dl) in diabetic groups

Figure 10a: Serum triglycerides level (mg/dl) in obese groups

Figure 10b: Serum triglyceride level (mg/dl) in diabetic groups

Figure 11a: Atherogenic index of plasma (AIP) in obese groups

Figure 11b: Atherogenic index of plasma (AIP) in diabetic groups

Figure 12a: Nitrite/ Nitrate level (µmol/L) in obese groups

Figure 12b: Nitrite/ Nitrate level (µmol/L) in diabetic groups

a, b, c and d Denote statistical significance at p ≤ 0.05.
a Vs control G., b Vs control obese G., c Vs irisin treated obese G. and d Vs exercise treated obese G.

a, b, c and d Denote statistical significance at p ≤ 0.05.
a Vs control G., b Vs control diabetic G., c Vs irisin treated diabetic G. and d Vs exercise treated diabetic G.
Correlation between serum irisin level (ng/ml) and other parameters in obese and diabetic groups:
Figures (13 and 14) showed that; serum irisin level is negatively correlated with body weight, body mass index, serum insulin, serum fasting blood sugar, insulin resistance, serum osteopontin, serum triglycerides and atherogenic index. While it is positively correlated with insulin sensitivity, serum HDL cholesterol level and serum nitrite/nitrate level in both obese and diabetic groups.

Figure (13):- Correlation between serum irisin level (ng/ml) and other parameters in obese groups.
Discussion:-
The adverse effects of obesity and diabetes have been extensively studied in experimental animals. Low dose streptozotocin (STZ) and a high fat diet (HFD) are an ideal animal model for type 2 diabetes. This model simulates the natural disease progression and metabolic characteristics typical of type 2 diabetes. Treatment with the β-cell toxin STZ results in a severe reduction in functional β-cell mass. This model mimics the pathology of type 2 diabetes on a shorter time scale than found in the human condition (Skovsø 2014).

It is evident from the results of our work that non-treated obese and diabetic groups showed significant increase in body weight and body mass index comparing to control group. This rise in the mentioned parameters may be attributed to the high caloric diet (contain 60% fat) and positive energy balance leading to augmented mass of body fat and fat accumulation in adipose tissue (Hashem et al. 2018).

In contrast, treated obese and diabetic groups by irisin showed significant decrease in body weight and body mass index comparing to control obese group and control diabetic group respectively, which is consistent with previous
It has been established that irisin plays a significant role in energy metabolism and glucose tolerance. After being released from muscle following exercise or externally injected, it circulates and exerts its function as a hormone that stimulates the browning of white adipose tissue which translates into burning more calories and therefore increasing oxygen consumption and thermogenesis in adipocytes (Pardo et al., 2014). Also, irisin increase uncoupling protein 1 (UCP1) which is a key protein to the thermogenic capacity of adipose tissues that enables the separation of lipid oxidation from ATP production, allowing a higher metabolic rate and the conversion of nutritional energy to heat (Xiong et al. 2015).

Treated obese and diabetic groups by moderate intensity swimming exercise showed significant decrease in body weight and body mass index comparing to control obese group and control diabetic group respectively, which is consistent with previous studies (Seo et al., 2014). The reduction of the anthropometric parameters in the exercise treated groups is mainly attributable to exercise-induced energy consumption (Lu et al., 2016).

The exogenous administration of irisin has the same effect of exercise. Hence, obese individuals who cannot lose weight with or without exercise due to various conditions may potentially benefit from this exogenous administration of irisin (Ercan et al., 2018).

Eight weeks consumption of HFD induce hyperinsulinemia and altered glucose homeostasis due to insufficient compensation by the beta cells of the pancreatic islets (Czech 2017). Non treated obese and diabetic groups showed that; serum insulin level, serum fasting glucose level, insulin resistance (HOMA-IR) significantly increased and homeostatic model assessment of insulin sensitivity (HOMA-S) significantly decreased comparing to control group. Obesity and diabetes are associated with a chronic low-grade pro-inflammatory metabolic state that stimulate signals from specialized cells (adipocytes, macrophages, and T-cells) and initiate the inflammatory responses that induce alterations in metabolic homeostasis through the secretion of numerous cytokines and adipokines (Guilherme et al. 2012).

On the other hand, treated obese and diabetic groups by irisin showed that; serum insulin level, serum fasting glucose level, insulin resistance (HOMA-IR) significantly decreased and homeostatic model assessment of insulin sensitivity (HOMA-S) significantly increased comparing to control obese group and control diabetic group respectively. These results are consistent with previous studies (Moreno-Navarrete et al. 2013) that prove the effect of irisin therapy in preventing the occurrence of DM by reducing the risk of insulin resistance, lowering serum glucose and reducing the weight in rats which are under HFD. Irisin administration result in improvement in glucose homeostasis which was achieved by increased glucose uptake combined with reduced gluconeogenesis in the liver (Pósa et al., 2015).

The results of exercise treated groups are nearly similar to irisin treated groups and this proves that irisin mimics the exercise effect in the improvement of obesity related disorders, which is consistent with (Basil et al., 2014). Exercise prevent chronic low-grade inflammatory state and decrease secretion of TNF-alpha and increase level of IL-10 thus preventing the deleterious effect on glucose homeostasis (Guilherme et al., 2012). The effect of exercise also, may be attributed to an increased expressionand/or activity of certain proteins involved in glucose metabolism and insulin signaling in skeletal muscles such as glycogen synthase and glucosetransporter 4 (GLUT4) (Moustafa and Marwa 2017) (Ercan et al., 2018).

There is significant decrease in serum irisin level in nontreated obese and diabetic groups in comparison with control group. Our results are in consistent with (Yang et al., 2015) they showed significant negative correlation between obesity and serum irisin level. Lower levels of circulating irisin in obese and T2DM could be explained by impaired (PGC-1a) expression and functions in the muscle and adipose tissue (Huh et al., 2012).

The groups received exogenous irisin (obese and diabetic) or exercised showed increased levels of serum irisin. Serum levels were higher in the exercise groups compared with the exogenous irisin injected groups, but the difference did not reach statistical significance. These results are consistent with some authors, who found that chronic exercise induce a significant increase in serum irisin level in the exercised rats when compared with sedentary ones (Ercan et al. 2017).
Osteopontin (OPN) plays an important role in infiltration and accumulation of macrophages in adipose tissue in the early stages of obesity and increased macrophage-related inflammatory activity could be a link between inflammation and insulin resistance (Kamei et al. 2006). The results of our work showed that, the level of OPN is significantly increased in obese and type II diabetic rats as compared to control group. Our results are consistent with previous work of (Ahmad et al., 2013).

In contrast, the treated obese and diabetic groups by irisin showed significant reduction in serum OPN level compared to control obese and diabetic groups. Also treated obese and diabetic groups by moderate intensity swimming exercise showed the same results as treated irisin groups.

Blood lipids disorders are critical risk factors for cardiovascular diseases. Lipid abnormalities, often termed “dyslipidemia”. Lipid abnormalities are common in obese and diabetic (Bhowmik et al., 2018).

In our study we found that serum triglycerides leveland atherogenic index of plasma in obese and diabetic groups was significantly higher than control group. In contrast serum HDL cholesterol level in obese and diabetic groups was significantly lower than control group. Our results are similar to previous studies reporting that HFD and induced type 2 diabetes cause dyslipidemia in rodents (Gao et al., 2018). The hepatocyte expression of transcription factors sterol regulatory element binding protein-1c (SREBP-1c) and nuclear factor-κB (NFκB), and their target genes found to be upregulated in HFD fed obese and diabetic rats compared to healthy animals. Upregulation of these factors regulate fatty acid and cholesterol metabolism genes (Khadke et al., 2019).

Treated obese and diabetic groups by irisin showed that; serum triglycerides level and atherogenic index of plasma significantly reduced comparing to control obese group and control diabetic group respectively. In contrast serum HDL cholesterol level in irisin treated group significantly increased comparing to control obese group and control diabetic group. In adipocytes, irisin enhanced basal lipolysis which prevented by inhibition of adenylate cyclase or protein kinase A (PKA); irisin increased hormone-sensitive lipase (HSL) expression and phosphorylation; it increased PKA activity, and cAMP and HSL mRNA levels, but reduced perilipin expression. These results indicate that irisin ameliorates lipid metabolic derangements. So, irisin enhance lipolysis via cAMP–PKA–HSL/perilipin pathway. Perilipin is a protein localized on the surface of lipid droplets that serves as a gatekeeper and inhibits lipolysis. The phosphorylation of HSL causes the activation of HSL, and the phosphorylation of perilipin induces the translocation of HSL from the cytosol to the surface of lipid droplets for converting stored triglycerides to glycerol and free fatty acids (FFAs). Irisin-induced down-regulation of perilipin and up-regulation of HSL promote the lipolysis (Xiong et al., 2015).

Also treated obese and diabetic groups by moderate intensity swimming exercise showed that; serum triglycerides level and atherogenic index of plasma significantly reduced comparing to control obese group and control diabetic group respectively. In contrast serum HDL cholesterol level in exercise treated group significantly increased comparing to control obese and diabetic groups. Also treated obese and diabetic groups by moderate intensity swimming exercise showed the same results as treated irisin groups.

In present study nitrite/nitrate are significantly reduced in obese and diabetic rats compared to control group. Decreased nitric oxide (NO) signaling due to reduced endogenous formation or increased metabolism is a risk factor for development of obesity and diabetes and associated comorbidities. The mechanisms that explain this pathological alteration are reduced nitric oxide (NO) production and low NO bioavailability. Diminished levels of NO in obese and diabetic states may be due to decreased expression of nitric oxide synthase (NOS), impairments in NOS activity, or by the reaction of NO with reactive species. Decreased NOS expression commonly occurs in obese and diabetic states (Sansburyand Hill 2014).

Treated obese and diabetic groups by irisin shows significant increase in serum nitrate level in comparison with control obese and diabetic groups respectively. These results are consistent with previous study (Fang et al., 2015).
The mechanism for this increase in serum nitrate level seemed to be related to AMP-activated protein kinaseendothelial NO synthase (AMPK-eNOS) signaling pathway activation. High FFA levels released from excess white adipose tissue could impair eNOS phosphorylation through inhibiting this signaling pathway in obesity. Irisin turn white adipocytes into brown adipocytes and reduce circulating FFA levels from adipose tissue and prevent the effect of FFA (Wang et al., 2012). Thus, irisin plays an important role in increasing NO production and activating the AMPK- Akt- eNOS pathway (Fu et al., 2016).

Also, treated obese and diabetic groups by moderate intensity swimming exercise have the same results of significant increase in serum nitrate level compared to control obese and diabetic groups as irisin treated. Nitric oxide production increased in skeletal muscle in response to physical activity. Moderate exercise increasing activity of the entire NOS pool (the cumulative activity of endothelial NOS, neuronal NOS, and inducible NOS). Enhanced NO release in physical exercise is mediated through eNOS gene transcription (Dyakova et al., 2015).

In our worktreated obese and diabetic groups by combination of both irisin and exercise showed a significant change compared to irisin treated groups and exercise treated groups. This was noticed in all parameters except in body weight gain and body mass index. There is no significant difference between the results when using this regimen of combination and control group. This effect may be due to the potentiation between the effect of irisin and the effect of exercise.

Conclusion:
Irisin and exercise showed anti hyperglycemic, anti-hyperlipidemia, antioxidant effect with potentiation effect when used together. This study recommended that the use of irisin is promising and deserve further investigation and evaluation in clinical practice in treating and prevention of metabolic syndrome

References:
1. Abd Al-Aleem D. and Moursi S. (2017): Effect of Exercise on the Interplay between Serum Irisin and Some Metabolic and Hemostatic Parameters in Obesity Rat Model. Med. J. Cairo Univ., 85, (5): 1653-1667.
2. Ahmad R., Al-Mass A., Al-Ghawas D., Shareif N., Zghoul N., et al. (2013): Interaction of Osteopontin with IL-18 in Obese Individuals: Implications for Insulin Resistance. PLoS One.; 8(5): e63944.
3. Alencar J.P., Prado F.M., Coelho M.B., Raissa M.R., José A.N., and Ivana R.R. (2017): Low Irisin Levels in Patients with Type 2 Diabetes Mellitus without Current Treatment: a Systematic Review. International archives of Medicine section: endocrinology ISSN: 1755-7682 Vol. 10 No. 171 and endothelial function in lean and obese subjects. Clin Endocrinol (Oxf) 83: 339–343.
4. Basil O., Maysaa J. and Ghassan M. (2014): Irisin impact as a medication that ameliorate and hinder the development of insulin resistance associated disorders without regular exercise (experimental study). Journal of Dental and Medical Sciences. 13,(9): 28-35.
5. Bhowmik, B., Siddiquee T., Mujumder A., Afsana F., Ahmed T., Mdala A., et al. (2018): Serum lipid profile and its association with diabetes and prediabetes in a rural bangladeshi population. international journal of environmental research and public health,15(9), 1944.
6. Bird R. and Hawley A. (2017): Update on the effects of physical activity on insulin sensitivity in humans. BMJ open sport & exercise medicine, 2(1), e000143.
7. Boström P., Wu J., Jedrychowski P., Korde A., Ye L., et al. (2012): A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis, Nature481, 463–468.
8. Chen C. A., Druhan L. J., Varadharaj S., Chen Y. R. and Zweier J. L. (2008): Phosphorylation of endothelial nitric-oxide synthase regulates superoxide generation from the enzyme. The Journal of biological chemistry, 283(40), 27038–27047.
9. Choi Y.K., Kim M.K., Bae K.H., Seo H.A., Jeong J.Y., Lee W.K., Kim J.G., Lee I.K. and Park K.G.( 2013): irisin levels in new-onset type 2 diabetes. Diabetes Research; p. 96-101.
10. Czech M. P. (2017): Insulin action and resistance in obesity and type 2 diabetes. Nature medicine, 23(7), 804–814.
11. da Rocha G. L., Crisp A. H., de Oliveira M. R., da Silva C. A., Silva J. O., et al. (2016): Effect of High Intensity Interval and Continuous Swimming Training on Body Mass Adiposity Level and Serum Parameters in High-Fat Diet Fed Rats. TheScientificWorldJournal, 2016, 2194120.
12. Dobiásová M. (2004): Atherogenic Index of Plasma [log(triglyceride/HDL-Cholesterol)]: Theoretical and Practical Implications. Clin. Chem., 50: 1113-1115.
13. Dyakova E. Y., Kapilevich L. V., Shylko V. G., Popov S. V. and Anfinogenova Y. (2015): Physical exercise associated with NO production: signaling pathways and significance in health and disease. Frontiers in cell and developmental biology, 3, 19.
14. Erkan B., Umit Z., Ebru G. G., Bahar Y. O., Faruk C., et al. (2018): Effects of Irisin and Exercise on Metabolic Parameters and Reproductive Hormone Levels in High-Fat Diet-Induced Obese Female Mice. Reproductive Sciences, Vol. 25(2): 281-291.
15. Fang H., Shuxian Z., Ningning H., Di W. and Xiaodong S. (2015): Irisin improves endothelial function in obese mice through the AMPK-eNOS pathway. Am J Physiol Heart Circ Physiol, 309: H1501–H1508.
16. Fu J., Han Y., Wang J., Liu Y., Zheng S., Zhou L., Jose P.A. and Zeng C. (2016): Irisin Lowers Blood Pressure by Improvement of Endothelial Dysfunction via AMPK-Akt-eNOS-NO Pathway in the Spontaneously Hypertensive Rat. J Am Heart Assoc. 26;5(11): e003433.
17. Gao L., Lin Z. Liu Y., et al. (2018): Hypolipidemic effect of FragarianilgerrensisSchlecht. medicine compound on hyperlipidemic rats; Lipids in Health and Disease.17: 222.
18. Ghanbari E., Nejati V. and Khazaei, M. (2016): Improvement in Serum Biochemical Alterations and Oxidative Stress of Liver and Pancreas following Use of Royal Jelly in Streptozotocin-Induced Diabetic Rats. Cell journal, 18(3): 362–370.
19. Grove T.H. (1979): Effect of reagent pH on determination of high-density lipoprotein cholesterol by precipitation with sodium phosphotungstate-magnesium. Clin. Chem., 25 (4): 560-564.
20. Guilherme F. F. S., Marisa C. R., Fernanda O. D., et al. (2012): The effects of exercise modalities on adiposity in obese mice. Clinics, 67(12): 1469-1477.
21. Han F., Zhang S., Hou N., Wang D. and Sun X. (2015): Irisin improves endothelial function in obese mice through the AMPK-eNOS pathway. Am J Physiol Heart Circ Physiol.:309:H1501–H1508.
22. Hashem M., Nasr El-Deen N. and Ghareeb O. (2018): Biochemical Effects Of Ginger And/or Green Tea Extracts In High Fat Diet-Induced Obese Rats. Sow Vet Res; 55:241-249.
23. He F., Li J., Liu Z., Chuang C. C., Yang W. and Zuo L. (2015): Redox Mechanism of Reactive Oxygen Species in Exercise. Frontiers in physiology, 7: 486.
24. He W., Bai Q., La A., Tang C. and Zang A. (2015): Irisin levels are associated with urotensin levels in diabetic patients. J Diabetes Invest Vol. 6 No.5, 362.
25. Huh J.Y., Panagiotou G., Mougios V., Brinkoetter M., Vamvini M.T., Schneider B.E. and Mantzoros C.S. (2012): FNDC5 and irisin in humans: I. Predictors of circulating concentrations in serum and plasma and II. mRNA expression and circulating concentrations in response to weight loss and exercise. Metabolism, 61:1725–1738.
26. Hye J. L., Jung O. L., Nami K., et al. (2015): Irisin, a Novel Myokine, Regulates Glucose Uptake in Skeletal Muscle Cells via AMPK. Molecular Endocrinology, 29, (6): 873–881.
27. Irving, B.A., Still, C.D. and Argyropoulos, G. (2014): Does IRISIN Have a BRITE Future as a Therapeutic Agent in Humans?. Curr Obes3(2): 235–241.
28. Kähles F., Findeisen H. M. and Brummer D. (2014): Osteopontin: A novel regulator at the cross roads of inflammation, obesity and diabetes. Molecular metabolism, 3(4): 384–393.
29. Kamei N., Tobe K., Suzuki R., Ohsugi M., Watanabe T., et al. (2006): Overexpression of monocyte chemoattractant protein-1 in adipose tissues causes macrophage recruitment and insulin resistance. J Biol Chem., 281(36): 26602-26614.
30. Kao P.C., Taylor R.L. and Service F.J. (1994): Proinsulin by Immunochemiluminometric Assay for the Diagnosis of Insulinoma. Jouranl of Clin Endocrinol Metab; 78 (5): 1048-1051.
31. Khadke S.P., Kuvalakar A.A., Harsulkar A.M. and Mantri N. (2019): High Energy Intake Induced Overexpression of Transcription Factors and Its Regulatory Genes Involved in Acceleration of Hepatic Lipogenesis: A Rat Model for Type 2 Diabetes. Biomedicines, 7(4):76.
32. Kiefer F.W., Zeya M., Todoric J., Huber J., Geyeregger R., Weichhart T., et al. (2008): Osteopontin expression in human and murine obesity: Extensive local up-regulation in adipose tissue but minimal systemic alterations. Endocrinology, 149: 1350–1357.
33. Kohl T.O. and Ascoli C.A. (2017): Immunometric Double-antibody Sandwich Enzyme-Linked Immunosorbent Assay. Cold Spring Harb Protoc.:2017(6):pdb.Prot093724.
34. Korta P., Pocheć E. and Mazur-Bialy A. (2019): Irisin as a Multifunctional Protein: Implications for Health and Certain Diseases. Medicina.; 55(8): 485.
35. Kyung H., Park, L. Z., Mary B., Bindiya T., Ayse ., Kyoung E. J., Michael A. T., Eleni V. G.i, Joo Y. H., Fadime D., Cynthia R. D., Judith A. C., Christos S. M.(2013): Circulating Irisin in Relation to Insulin Resistance and the Metabolic Syndrome. The Journal of Clinical Endocrinology & Metabolism, Volume 98, Issue 12, Pages 4899–4907
36. Liu J.J., Wong M.D., Toy W.C., Tan C.S., Liu S., Ng X.W., Tavintharan S., Sum C.F., Lim S.C.(2013): Lower circulating irisin is associated with type 2 diabetes mellitus. J Diabetes Complications.;27(4),365–369.
37. Liu S., Du F., Li X., Wang M., Duan R., Zhan J., Wu Y. and Zhang Q. (2017): Effects and underlying mechanism of irisin on the proliferation and apoptosis of pancreatic β cells. PLoS ONE 12(4): e0175498.
38. Liu T.Y., Shi C.X., Gao R., Sun H.J., Xiong X.Q., Ding L., et al. (2015): Irisin inhibits hepatic gluconeogenesis and increases glycogen synthesis via the PI3K/Akt pathway in type 2 diabetic mice and hepatocytes. Clin Sci (Lond.); 129: 835–850.
39. Lu Y., Li H., Shen S. W., Shen Z. H., Xu M., Yang C. J., et al. (2016): Swimming exercise increases serum irisin level and reduces body fat mass in high-fat-diet fed Wistar rats. Lipids in health and disease, 15: 93.
40. Matthews D.R., Hosker J.P., Rudensis A.S., Naylor B.A., Treacher D.F. and Turner R.C. (1985): Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia; 28:412-19.
41. Mazur-Bialy A.I., Bilski J., Pochec E. and Brzozowski T.(2017): New insight into the direct anti-inflammatory activity of a myokine irisin against proinflammatory activation of adipocytes. Implication for exercise in obesity. J PhysiolPharmacol. 68(2):243-251.
42. Mcgowan M.W., Artiss J.D., Strandbergh D.R. and Zak B., (1983): A peroxidase-coupled method for the colorimetric determination of serum triglycerides.Clin Chem; 29 (3):538-542.
43. Mehrabian S., Taheri E., Karkhanem M., Qorbani M. and Hosseini S. (2016): Association of circulating irisin levels with normal weight obesity, glycemic and lipid profile. Journal of Diabetes & Metabolic Disorders (15:17)
44. Montgomery H.A. and Dymock J.F. (1961): Colorimetric determination of nitric oxide. In Analyst; 86:414.
45. Moreno-Navarrete J.M., Ortega F., Serrano M., Guerra E., Pardo G., Tinahones F., et al.(2013): Irisin is expressed and produced by human muscle and adipose tissue in association with obesity and insulin resistance. J Clin Endocrinol Metab.; 98(4): 769–778.
46. Moustafa H. and Marwa A. (2017): Effect of Swimming Exercise on Irisin Levels in Streptozotocin Induced Type 1 and Type 2 Diabetes Mellitus in Rats.Med. J. Cairo Univ., Vol. 85, No. 7, December: 2625-2634.
47. Pandey G., Shihabudeen M. S., David H. P., Thirumurugan E. and Thirumurugan K. (2015): Association between hyperleptinemia and oxidative stress in obese diabetic subjects. Journal of diabetes and metabolic disorders. (14)14-24.
48. Pardo M., Crujeiras A. B., Amil M., et al. (2014): Association of irisin with fat mass, resting energy expenditure, and daily activity in conditions of extreme body mass index. International Journal of Endocrinology.;2014: 857270.
49. Pedersen B.K., Febbraio M.A.(2012): Muscles, exercise and obesity: skeletal muscle as a secretory organ. Nature Reviews. Endocrinology; 8:457–465.
50. Pósa A., Szabó R., Kupai K., Csonka A., Szalai Z., Veszelka M., et al. (2015): Exercise training and calorie restriction influence the metabolic parameters in ovariectomized female rats. Oxid Med Cell Longev., 2015:787063.
51. Sabitha V., Ramachandran S., Naveen K.R. and Panneerselvam K.(2011): Antidiabetic and antihyperlipidemic potential of Abelmoschus esculentus (L.) Moench. In streptozotocin induced diabetic rats. J Pharm Bioallied Sci:397–402.
52. SansburyB.E. and Hill B.G. (2014):Regulation Of Obesity And Insulin Resistance By Nitric Oxide. Free Radic Biol Med. 73: 383–399.
53. Seo D., Kwak H., Lee S., Cho Y., Song I., Kim N., Bang H., Rhee B., Ko K., Park B. and Han J. (2014): Effects of aged garlic extract and endurance exercise on skeletal muscle FNDC-5 and circulating irisin in high-fat-diet rat models. Nutr. Res. Pract., 8: 177-82,
54. Shaw J., Sicree R. and Zimmet P. (2010): Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract.; p 4-14.
55. Skovnø S. (2014): Modeling type 2 diabetes in rats using high fat diet and streptozotocin. Journal of diabetes investigation, 5(4): 349–358.
56. Sommermatter S., Shui G., Maag D., Santos G., Wenk M. R. and Handschin C. (2013): PGC-1α improves glucose homeostasis in skeletal muscle in an activity-dependent manner. Diabetes, 62(1): 85–95.
57. Tietz N.W., (1986): Determination of blood glucose. Text book of clinical chemistry WB Saunders, Philadelphia; 796.

58. Vincent V., Thakkar H., Aggarwal S., Mridha A. R., Ramakrishnan L. and Singh, A. (2019): ATP-binding cassette transporter A1 (ABCA1) expression in adipose tissue and its modulation with insulin resistance in obesity. Diabetes, metabolic syndrome and obesity: targets and therapy, 12: 275–284.

59. Wang B., Yu Y. and Han L. (2012): Adiponectin improves endothelial dysfunction caused by elevated FFAs levels, partially through cAMP-dependent pathway. Diabetes Res Clin Pract 97: 119–124.

60. Wang Y. and Xu D. (2017): Effects of aerobic exercise on lipids and lipoproteins. Lipids in health and disease, 16(1): 132.

61. Xiong X.Q., Chen D., Sun H.J., Ding L., Wang J.J., Chen Q., et al. (2015): FNDC5 overexpression and irisin ameliorate glucose/lipid metabolic derangements and enhance lipolysis in obesity. Biochim et Biophysica Acta.1852 (9):1867–1875.