Effect of Exercise on the Interplay between Serum Irisin and Some Metabolic and Hemostatic Parameters in Obesity Rat Model

DALIA I. ABD AL-ALEEM, M.D. and SUZAN M.M. MOURSI, M.D.
The Department of Physiology, Faculty of Medicine, Zagazig University, Egypt

Abstract

Background: Abnormalities in coagulation and hemostasis represent a well-known link between obesity and thrombosis. Irisin is a novel myokine and adipocytokine. There are apparently contradictory reports concerning irisin levels and their correlation with obesity-related parameters. Moreover, the effect of exercise training on irisin level remains controversial.

Objective: This study was designed to investigate irisin level in case of high fat diet induced obesity in rats and to evaluate effect of chronic exercise training on this level in relation to some metabolic and hemostatic parameters.

Material and Methods: Thirty adult male albino rats were equally divided to 3 groups, group (1): Control group, group (II): High Fat Diet (HFD) fed group for 8 weeks (58% fat) and group (III): High fat diet fed group and exposed to a moderate exercise training protocol (swimming for 90min/day for 5 days weekly) for 8 weeks. In all groups, BMI, Abdominal Circumference (AC), serum levels of irisin, glucose, insulin, HOMA-IR, lipid profile parameters, TNF-α, MDA, plasma fibrinogen and d-dimers levels were measured and Bleeding Time (BT), Whole Blood Clotting Time (WBCT), PT, aPTT were also evaluated.

Results: The present study revealed that HFD for 8 weeks significantly increased BMI, AC, serum glucose, insulin, Total Cholesterol (TC), Triglycerides (TG), Very Low Density Lipoproteins (VLDL), Low Density Lipoproteins (LDL), TNF-α, Malondialdehyd (MDA) levels and HOMA-IR, plasma fibrinogen and d-dimers levels. Furthermore, there was a significant reduction in serum irisin level in the same group (group II), with a significant negative correlation between this hormone and all the above mentioned parameters, except, there was a significant positive correlation with the decreased levels of High Density Lipoproteins (HDL). Further, there was a significant reduction in BT, WBCT, PT, aPTT (with a significant positive correlation versus serum irisin level). However, exercise training in group III produced a significant increase in serum irisin level and marked improvement in the above mentioned metabolic and hemostatic parameters with the preservation of the same type of correlations versus serum irisin level in the exercising group.

Conclusion: Elevated irisin levels by chronic exercise training may play a role in improving the metabolic and hemostatic abnormalities associated with obesity in rats.

Key Words: Obesity – Insulin – Exercise – Hemostasis – Irisin.

Introduction

OBESITY is a worldwide health problem, associated with a wide range of diseases such as diabetes, insulin resistance, cardiovascular disease, chronic kidney disease, cancer and other metabolic syndromes which leads to serious threat to human health [1].

Abnormalities in coagulation and haemostasis represent a well-known link between obesity and thrombosis, involving elevated expression of the prothrombotic molecules and increased platelet activation [2].

Moreover, obesity characterized by a chronic low-grade inflammation with increased levels of the pro-inflammatory cytokines that may indirectly cause thrombosis by inducing oxidative stress and endothelial dysfunction [3].

On the other hand, chronic aerobic exercise training may cause favorable adaptations that could contribute to decreased risk for ischemic events both at rest and during physical exertion [4].

Irisin, a novel myokine secreted by skeletal muscle as a hydrolyzed fragment of fibronectin type III domain-containing protein 5 (FNDC5) [5,6], which is reported to be a peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1 α)-dependent hormone [7].

Also, it has been shown that irisin can be expressed and secreted by adipose tissue [8]. However,
there are apparently contradictory reports addressing irisin levels and their correlation with body mass and obesity-related parameters.


Moreover, the effect of exercise on circulating irisin level is still under debate. Some investigators reported higher irisin levels after training for 3 weeks in mice [12]. However, other researchers found that exercise training does not affect skeletal muscle FNDC-5 or circulating irisin level [13].

This study was designed to investigate irisin level in case of high fat diet induced obesity in rats and to evaluate effect of chronic exercise training on this level in relation to some metabolic and hemostatic parameters.

Material and Methods

Thirty adult male albino rats weighing 179-212gm, were obtained from Faculty of Medicine Animal House-Zagazig University and the animal experiments were conducted during summer 2016 and approved by the local ethics committee. They were fed the commercial rodent chow containing: 25.8% protein, 62.8% carbohydrate and 11.4% fat (a total 12.6kJ/g) with free access to water [14], but the rats in high fat-fed groups received high-fat chow containing: 16.4% protein, 25.6% carbohydrate, and 58.0% fat (a total 23.4kJ/g) in the form of cotton seed oil added to the laboratory chow diet [15], those diets were obtained from Faculty of Agriculture, Zagazig University.

Rats kept at room temperature and were maintained on a 12h light/dark cycle. Rats were adapted for one week before the start of the experiment.

Rats were randomly assigned to three equal groups (n=10):

Group (I): Control rats fed a standard chow for 8 weeks.

Group (II): High Fat Diet (HFD) fed rats for 8 weeks without exercise training.

Group (III): High Fat Diet (HFD) fed rats with a moderate exercise training protocol for 8 weeks.

Exercise training protocol: The rats in the training group subjected to a swimming exercise in a barrel filled with water to a depth of 40-50cm at 33-35°C [16]. Training duration was 90min swimming within each training day during 5 training days of a week for 8 weeks [17].

Initial and final BMI for all rats were calculated and final abdominal circumferences were evaluated. Moreover, bleeding time was evaluated before blood sampling at the end of the experiment.

Anthropometric measures:

Measuring body weight: By using a digital balance (Germany).

Measuring rat length: Nose to anus length was measured according to Novelli et al., [18].

Measuring Abdominal Circumference (AC): A plastic tape was used to measure the waist circumference at the largest zone of the rat's abdomen [19].

Calculating BMI index via this equation: Body weight (gm)/length $^2$ (cm$^2$), where the cut off value of obesity BMI is more than 0.68gm/cm$^2$ [18].

Sample collection: Retro-orbital venous plexus blood samples were collected and divided into three vials. The first one was containing 3.2% sodium citrate in the ratio 1:9 with the blood and centrifuged at 2000rpm for 15min. Plasma obtained used for estimation of Prothrombin Time (PT), activated Partial Thromboplastin Time (aPTT), plasma d-dimers and fibrinogen levels. Serum was obtained by allowing the blood samples to clot then centrifugation at 3000rpm for 20 minutes and kept at (−20°C) and used to measure levels of irisin, glucose, insulin, Total Cholesterol (TC), Triglycerides (TG), High Density Lipoproteins (HDL), Very Low Density Lipoproteins (VLDL), Low Density Lipoproteins (LDL), tumor necrosis factor-alpha (TNF-α) and Malondialdehyde (MDA). The remaining vial was filled with fresh blood for measuring the Whole Blood Clotting Time (WBCT).

Biochemical analysis:

1- Serum irisin level: using a (Irisin ELISA kit EK-067-16; Phoenix Pharmaceuticals, Burlingame, CA), on a spectrophotometric reader at a wavelength of 450 nm according to Yang et al., [17].

2- Serum glucose levels: Using enzymatic Kits (Biotechnology, Egypt), according to Tietz et al., [20].
3- Serum insulin levels: using rat ELISA kit, (Product Number: RAB0904, Sigma-Aldrich Chemie GmbH, U.S.A), according to Temple et al., [21].

Calculation of homeostasis model assessment of insulin resistance (HOMA-IR)= insulin (µIU/mL) x glucose (mg/dl)/405 [22].

4- Serum Total Cholesterol (TC) levels: Using rat ELISA kit, (Catalog Number: 2011-11-0198, Shanghai Sunred biological technology, China), according to Allain et al., [23].

5- Plasma fibrinogen levels: According to method of Quick, [31].

6- Serum d-dimers levels: Using ELIZA kit, (Catalog Number: RAB0480, provided by Sigma-Aldrich Co), according to the method described by Engvall and Perlmann [29].

Haemostatic parameters:

1- Bleeding time: According to method of Martin [30].

2- Whole blood clotting time: According to method of Quick, [31].

3- Prothrombin time: According to method of Arkin using coagulometer [32].

4- Activated partial thromboplastin time: According to method of Ansell [33] using coagulometer.

5- Plasma fibrinogen levels: According to method of Cooper and Douglas [34] using coagulometer.

6- Plasma d-dimers levels: Using ELIZA kit, (GenWay Biotech, Inc, ca 40-88-234402, USA) according to Declerck et al., [35].

Statistical analysis:

Results were presented as mean ± SD for and analyzed using Version 18 SPSS program (SPSS Inc, Chicago, IL, USA). One way Analysis of variance (ANOVA) was used followed by student-Least Significant Differences (LSD) test to compare statistical differences between groups. p-value less than 0.05 was considered to be significant. Pearson’s test was done to detect correlations between parameters.

Results

The present study showed that HFD significantly decreased serum irisin level (p<001). Moreover, it significantly increased BMI, AC, serum glucose, insulin and HOMA-IR, serum total cholesterol, triglyceride, LDL, VLDL, TNF-α, MDA and plasma fibrinogen and d-dimers levels (p<001) [with significant negative correlations versus serum irisin level (r=-0.734, p<0.05, r=-0.738, p<0.05, r=-0.904, p<0.001, r=-0.670, p<0.05, r=-0.865, p<0.01, r=-0.775, p<0.01, r=-0.863, p<0.01, r=-0.767, p<0.05, r=-0.753, p<0.05, r=-0.784, p<0.01 and r=-0.795, p<0.01 respectively], but significantly decreased serum HDL level, BT, WBCT, PT and aPTT (p<0.001) [with significant positive correlations versus serum irisin level (r=0.733, p<0.05, r=0.852, p<0.01, r=0.682, p<0.05, r=0.878, p<0.01 and r=0.745, p<0.05 respectively] (Tables 1-3).

Whereas, exercise intervention resulted in a significant recovery of serum irisin level (p<0.05). In addition, exercise intervention significantly decreased BMI, waist circumference, serum glucose, insulin and HOMA-IR, serum total cholesterol, triglyceride, LDL, MDA, plasma fibrinogen, plasma d-dimers (p<0.001), serum VLDL and TNF-α (p<0.01) levels in HFD with exercise intervention compared to HFD group. Furthermore, there were significant negative correlations between those measures and serum irisin levels (r=-0.634, p<0.05, r=-0.658, p<0.05, r=-0.799, p<0.01, r=-0.838, p<0.01, r=-0.867, p<0.01, r=-0.765, p<0.05, r=-0.741, p<0.05, r=-0.647, p<0.05, r=-0.703, p<0.05, r=-0.700, p<0.05, r=-0.674, p<0.05, r=-0.693, p<0.05 and r=-0.655, p<0.05 respectively). On the other hand, exercise intervention significantly increased HDL level, BT, WBCT (p<0.001), PT (p<0.01) and aPTT (p<0.05) with significant positive correlations versus serum irisin level (r=0.695, p<0.05, r=0.638, p<0.05, r=0.719, p<0.05, r=0.682, p<0.05 and r=0.735, p<0.05 respectively) in HFD with exercise intervention compared to HFD group (Tables 1-3).
Table (1): Serum irisin, body weights, BMI & AC of all studied groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>(n=10)</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum irisin (ng/ml)</td>
<td>X± SD</td>
<td>17.85±1.4</td>
<td>12.4±1.2</td>
<td>14.05±1.7</td>
</tr>
<tr>
<td>p-value of LSD</td>
<td></td>
<td>p&lt;0.001 a</td>
<td>p&lt;0.001 a</td>
<td>p&lt;0.001 a b</td>
</tr>
<tr>
<td>Initial BW (gm)</td>
<td>X± SD</td>
<td>196.7±9.1</td>
<td>193.6±11.1</td>
<td>196.2±10.4</td>
</tr>
<tr>
<td>p-value of LSD</td>
<td></td>
<td>NS a</td>
<td>NS a</td>
<td>NS b</td>
</tr>
<tr>
<td>Final BW (gm)</td>
<td>X± SD</td>
<td>242.3±10.55</td>
<td>397.1±18.55</td>
<td>302±15.18</td>
</tr>
<tr>
<td>p-value of LSD</td>
<td></td>
<td>r=–0.852, p&lt;0.01</td>
<td>r=–0.727, p&lt;0.05</td>
<td>r=–0.542, p&lt;0.05</td>
</tr>
<tr>
<td>Final BMI (gm/cm²)</td>
<td>X± SD</td>
<td>0.47±0.05</td>
<td>0.77±0.09</td>
<td>0.58±0.06</td>
</tr>
<tr>
<td>p-value of LSD</td>
<td></td>
<td>r=–0.967, p&lt;0.001</td>
<td>r=–0.734, p&lt;0.05</td>
<td>r=–0.634, p&lt;0.05</td>
</tr>
<tr>
<td>AC (cm)</td>
<td>X± SD</td>
<td>17.52±0.43</td>
<td>22.57±0.59</td>
<td>19.71±0.88</td>
</tr>
<tr>
<td>p-value of LSD</td>
<td></td>
<td>r=–0.800, p&lt;0.01</td>
<td>r=–0.738, p&lt;0.05</td>
<td>r=–0.658, p&lt;0.05</td>
</tr>
</tbody>
</table>

The results are expressed as mean ± SD.

a : Versus group I.
b : Versus group II.
NS : Non Significant (p>0.05).

Table (2): Metabolic parameters of all studied groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>(n=10)</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>X± SD</td>
<td>88.7±6.25</td>
<td>186.79±18.31</td>
<td>127±15.3</td>
</tr>
<tr>
<td>p-value of LSD</td>
<td></td>
<td>p&lt;0.001 a</td>
<td>p&lt;0.001 a</td>
<td>p&lt;0.001 a b</td>
</tr>
<tr>
<td>r with serum irisin</td>
<td></td>
<td>r=–0.915, p&lt;0.001</td>
<td>r=–0.904, p&lt;0.001</td>
<td>r=–0.799, p&lt;0.01</td>
</tr>
<tr>
<td>Insulin (µIU/ml)</td>
<td>X± SD</td>
<td>20.28±1.98</td>
<td>36.31±2.46</td>
<td>26.77±3.03</td>
</tr>
<tr>
<td>p-value of LSD</td>
<td></td>
<td>p&lt;0.001 a</td>
<td>p&lt;0.001 a</td>
<td>p&lt;0.001 a b</td>
</tr>
<tr>
<td>r with serum irisin</td>
<td></td>
<td>r=–0.824, p&lt;0.01</td>
<td>r=–0.670, p&lt;0.05</td>
<td>r=–0.838, p&lt;0.01</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>X± SD</td>
<td>4.46±0.70</td>
<td>16.82±2.61</td>
<td>8.47±1.8</td>
</tr>
<tr>
<td>p-value of LSD</td>
<td></td>
<td>p&lt;0.001 a</td>
<td>p&lt;0.001 a</td>
<td>p&lt;0.001 a b</td>
</tr>
<tr>
<td>r with serum irisin</td>
<td></td>
<td>r=–0.919, p&lt;0.001</td>
<td>r=–0.865, p&lt;0.01</td>
<td>r=–0.867, p&lt;0.01</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>X± SD</td>
<td>71.7±4.66</td>
<td>141.5±14.42</td>
<td>99.7±8</td>
</tr>
<tr>
<td>p-value of LSD</td>
<td></td>
<td>p&lt;0.001 a</td>
<td>p&lt;0.001 a</td>
<td>p&lt;0.001 a b</td>
</tr>
<tr>
<td>r with serum irisin</td>
<td></td>
<td>r=–0.764, p&lt;0.05</td>
<td>r=–0.775, p&lt;0.01</td>
<td>r=–0.765, p&lt;0.05</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>X± SD</td>
<td>42±3.91</td>
<td>87.89±10.41</td>
<td>69.89±5.64</td>
</tr>
<tr>
<td>p-value of LSD</td>
<td></td>
<td>p&lt;0.001 a</td>
<td>p&lt;0.001 a</td>
<td>p&lt;0.001 a b</td>
</tr>
<tr>
<td>r with serum irisin</td>
<td></td>
<td>r=–0.862, p&lt;0.01</td>
<td>r=–0.863, p&lt;0.01</td>
<td>r=–0.741, p&lt;0.05</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>X± SD</td>
<td>39.9±2.92</td>
<td>86±10.86</td>
<td>71.3±6.83</td>
</tr>
<tr>
<td>p-value of LSD</td>
<td></td>
<td>p&lt;0.001 a</td>
<td>p&lt;0.001 a</td>
<td>p&lt;0.001 a b</td>
</tr>
<tr>
<td>r with serum irisin</td>
<td></td>
<td>r=0.694, p&lt;0.05</td>
<td>r=0.733, p&lt;0.05</td>
<td>r=0.695, p&lt;0.05</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>X± SD</td>
<td>20.9±1.79</td>
<td>84.7±10.23</td>
<td>43.3±8.35</td>
</tr>
<tr>
<td>p-value of LSD</td>
<td></td>
<td>p&lt;0.001 a</td>
<td>p&lt;0.001 a</td>
<td>p&lt;0.001 a b</td>
</tr>
<tr>
<td>r with serum irisin</td>
<td></td>
<td>r=–0.817, p&lt;0.01</td>
<td>r=–0.767, p&lt;0.05</td>
<td>r=–0.647, p&lt;0.05</td>
</tr>
<tr>
<td>VLDL (mg/dl)</td>
<td>X± SD</td>
<td>8.51±1.04</td>
<td>17.93±1.67</td>
<td>15.25±2.02</td>
</tr>
<tr>
<td>p-value of LSD</td>
<td></td>
<td>p&lt;0.001 a</td>
<td>p&lt;0.001 a</td>
<td>p&lt;0.001 a b</td>
</tr>
<tr>
<td>r with serum irisin</td>
<td></td>
<td>r=0.658, p&lt;0.05</td>
<td>r=0.753, p&lt;0.05</td>
<td>r=0.703, p&lt;0.05</td>
</tr>
<tr>
<td>Serum TNFα (pg/ml)</td>
<td>X± SD</td>
<td>46.3±3.56</td>
<td>56.8±4.51</td>
<td>51±4.1</td>
</tr>
<tr>
<td>p-value of LSD</td>
<td></td>
<td>p&lt;0.001 a</td>
<td>p&lt;0.001 a</td>
<td>p&lt;0.001 a b</td>
</tr>
<tr>
<td>r with serum irisin</td>
<td></td>
<td>r=–0.817, p&lt;0.01</td>
<td>r=–0.637, p&lt;0.05</td>
<td>r=–0.700, p&lt;0.05</td>
</tr>
<tr>
<td>Serum MDA (nmol/ml)</td>
<td>X± SD</td>
<td>46.39±3.27</td>
<td>69.4±4.24</td>
<td>57.4±3.94</td>
</tr>
<tr>
<td>p-value of LSD</td>
<td></td>
<td>p&lt;0.001 a</td>
<td>p&lt;0.001 a</td>
<td>p&lt;0.001 a b</td>
</tr>
<tr>
<td>r with serum irisin</td>
<td></td>
<td>r=–0.861, p&lt;0.01</td>
<td>r=–0.823, p&lt;0.01</td>
<td>r=–0.674, p&lt;0.05</td>
</tr>
</tbody>
</table>

The results are expressed as mean ± SD.

a : Versus group I.
b : Versus group II.
NS : Non Significant (p>0.05).
Table (3): Haemostatic parameters of all the studied groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>(n=10)</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>BT (sec)</td>
<td></td>
<td>95.49±2.99</td>
<td>78.6±4.57</td>
<td>85.69±3.36</td>
</tr>
<tr>
<td></td>
<td>p-value of LSD</td>
<td>r=0.663, p&lt;0.05</td>
<td>p=0.001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>p=0.001&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>r with serum irisin</td>
<td>r=0.852, p&lt;0.01</td>
<td></td>
<td>p=0.638, p&lt;0.05</td>
</tr>
<tr>
<td>WBCT (sec)</td>
<td></td>
<td>120.8±16.09</td>
<td>72.6±10.95</td>
<td>95.6±9.43</td>
</tr>
<tr>
<td></td>
<td>p-value of LSD</td>
<td>r=0.899, p&lt;0.001</td>
<td>p=0.001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>p=0.001&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>r with serum irisin</td>
<td>r=0.682, p&lt;0.005</td>
<td></td>
<td>r=0.719, p&lt;0.05</td>
</tr>
<tr>
<td>PT (sec)</td>
<td></td>
<td>15.59±3.02</td>
<td>8.7±2.05</td>
<td>12.5±2.27</td>
</tr>
<tr>
<td></td>
<td>p-value of LSD</td>
<td>r=0.750, p&lt;0.05</td>
<td>p=0.001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>p=0.05, p&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>r with serum irisin</td>
<td>r=0.878, p&lt;0.01</td>
<td></td>
<td>r=0.682, p&lt;0.05</td>
</tr>
<tr>
<td>aPTT (sec)</td>
<td></td>
<td>23.2±4.04</td>
<td>16.9±1.96</td>
<td>20.3±6.65</td>
</tr>
<tr>
<td></td>
<td>p-value of LSD</td>
<td>r=0.878, p&lt;0.01</td>
<td>p=0.001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>p=0.05&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>r with serum irisin</td>
<td>r=0.745, p&lt;0.05</td>
<td></td>
<td>r=0.735, p&lt;0.05</td>
</tr>
<tr>
<td>Plasma fibrinogen (mg/dl)</td>
<td></td>
<td>227.2±14.93</td>
<td>321±33.23</td>
<td>273.3±19.32</td>
</tr>
<tr>
<td></td>
<td>p-value of LSD</td>
<td>r=0.721, p&lt;0.05</td>
<td>p=0.001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>p=0.001&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>r with serum irisin</td>
<td>r=0.784, p&lt;0.01</td>
<td></td>
<td>r=0.693, p&lt;0.05</td>
</tr>
<tr>
<td>Plasma D-dimer (mg/dl)</td>
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<td>59.8±4.82</td>
<td>94.2±10.37</td>
<td>76.2±8.05</td>
</tr>
<tr>
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<td>p-value of LSD</td>
<td>r=0.859, p&lt;0.01</td>
<td>p=0.001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>p=0.001&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>r with serum irisin</td>
<td>r=0.795, p&lt;0.01</td>
<td></td>
<td>r=0.655, p&lt;0.05</td>
</tr>
</tbody>
</table>

The results are expressed as mean ± SD.

<sup>a</sup> : Versus group I.
<sup>b</sup> : Versus group II.
NS : Non Significant (p>0.05).

Figs. (1-3): Correlation between serum irisin and final BMI in groups I, II & III respectively.

Figs. (4-6): Correlation between serum irisin levels and HOMA-IR in groups I, II & III respectively.

Figs. (7-9): Correlation between serum irisin and TNFα levels in groups I, II & III respectively.
Discussion

Central obesity has been linked to atherothrombotic events [36]. Exercise provides clear beneficial effects for the prevention of such diseases [37]. Some of the beneficial effects of exercise could be mediated by myokines like irisin hormone. However, controversy exists concerning irisin regulation and function [38].

The present study revealed that HFD for 8 weeks significantly increased BMI, AC, serum glucose, insulin and HOMA-IR, serum total cholesterol, triglyceride, LDL, VLDL, TNF-α, MDA and plasma d-dimers and fibrinogen levels (with a significant negative correlation versus serum irisin level), but significantly decreased serum HDL level, BT, WBCT, PT and aPTT (with a significant positive correlation versus serum irisin level).

In this work, there was a significant decrease in serum irisin level in HFD obese rats in comparison with that of control, whereas, exercise intervention resulted in a significant recovery of its level. In addition, BMI & AC were significantly decreased in HFD with exercise intervention compared to HFD group. Furthermore, there was a significant negative correlation between those measures and serum irisin levels in both groups. This is in agreement with previous reports [5,17,39].

Meanwhile, some contradictory results showed that obesity has a positive correlation with circulating irisin levels [11,40], while others have revealed no correlation between irisin and BMI [41,42]. Moreover, Seo et al., [13] founded that exercise training does not affect skeletal muscle FNDC-5 or irisin levels in obese rats. This controversy may be explained by variations in the types and durations of exercise used in the interventions or differences in the species used in the studies.

It has been shown that irisin can be expressed and secreted by adipose tissue [8,43]. It was also speculated that decreased circulating irisin level, due to down regulated FNDC5/irisin expression in adipose tissues, might contribute to muscle insulin resistance, speculating on the positive effects of circulating irisin on muscle insulin action [40,43].

Peroxisome proliferator-activated receptor-γ co-activator-1 α is a transcriptional co-activator up-regulated by exercise that can induce FNDC5 gene expression in the muscle [44].

Irisin has the capacity to stimulate the expression of thermogenic genes including uncoupling protein 1 that leads to browning of white adipose tissue and increase thermogenesis with subsequent reduction in body weight [44].

Central obesity is known to be associated with higher degree of cardiovascular risk [45-47]. Waist
circumference was evaluated in our study as a marker of central adiposity.

The significant decreases in bleeding and clotting times, PT and aPTT together with the significant increase in plasma d-dimers and fibrinogen levels in the obese rats in the present study are in accordance with the findings of El-Gendy and Abbas [48,49].

On the other hand, our study revealed that exercise significantly increased BT, WBCT, PT and aPTT times with a significant positive correlation with serum irisin level but significantly decreased plasma d-dimers and fibrinogen level with a significant negative correlation with serum irisin level in the HFD group with exercise intervention in comparison with that of HFD obese group.

Exercise has been reported to improve the metabolic profile and prevent atherogenesis, and it is supposed that irisin may mediate those effects [50].

Our study also revealed significant increases in circulating glucose and insulin together with HOMA-IR in obese rats. Moreover, those parameters were correlated negatively with irisin level that may be one of the mechanisms of the prothrombotic tendency observed in our study in HFD obese rats.

However, exercise intervention significantly decreased serum glucose, insulin and calculated HOMA-IR (which correlated negatively with irisin level) in obese exercising rats in comparison with that of obese sedentary group. Li et al., [51] also demonstrated that serum irisin concentrations were lower in type 2 diabetes mellitus patients and increased after continuous subcutaneous insulin.

The higher glucose levels cause posttranslational modifications of plasminogen leading to impaired fibrinolysis and activate adipose tissue macrophages [52]. Moreover, down regulation of phosphoinositide 3-kinase pathway enhances tissue factor expression as a result of insulin resistance in diabetic patients [53].

Exercise reduces hepatic glucose output via increasing insulin sensitivity in the liver of obese diabetic animals. This action is mediated by increased phosphorylation of Foxo1, which suppresses Phosphoenolpyruvate carboxykinase and glucose-6-phosphatase (G6Pase) [54].

Irisin also has a relevant action in alleviation of metabolic disorder by improvement of whole-body aerobic capacity and glucose metabolism [55]. It suppresses gluconeogenesis by G6Pase downregulation via phosphoinositide 3-kinase (PI3K)/Akt (Protein kinase B) pathway. Furthermore, Irisin increases glycogen Synthase (GS) activation [56].

Moreover, dyslipidemia, manifested by significant increases in total cholesterol, triglyceride, LDL, VLDL (with a significant negative correlation versus serum irisin level), and a significant reduction in HDL (with a significant positive correlation versus serum irisin level); can be added to the mechanisms of prothrombotic tendency observed in HFD obese rats.

High levels of circulating free fatty acids as a result of excess lipolysis in obese insulin-resistant cases are thought to increase the Tissue Factor (TF) expression [57]. Leptin "the satiety hormone made by adipose cells", can also increase TF in through Janus kinase pathway [58].

Meanwhile, exercise intervention significantly decreased total cholesterol, triglyceride, LDL, VLDL (with a significant negative correlation versus serum irisin level), but significantly increased HDL (with a significant positive correlation versus serum irisin level) in HFD with exercise intervention in comparison with that of HFD group. These results suggest that exercise intervention may be linked to the recovery of HFD-induced metabolic disorders [17].

Apart from the browning of adipose tissue, irisin was also shown to induce fatty acid uptake, increase oxidative metabolism, Peroxisome proliferator-activated receptor-gamma coactivator 1alpha (PGC-1 α) and even irisin itself in skeletal muscle itself [59,60]. It has been also shown that it ameliorates the dysregulation of hepatic glucose/lipid metabolism and cell death in insulin-resistant states via adenosine monophosphate kinase (AMPK) & and acetyl-CoA-carboxylase activation in hepatic cells [61].

Moreover, Jiang et al., [62] reported the relaxant effect of irisin on mesenteric arteries by nitric oxide release and also by inhibiting Ca \(^{2+}\) influx through blocking voltage gated calcium channels. irisin also activates endothelial nitric oxide synthase in aortas of HFD-obese mice [63].

The present results revealed a significant increase in TNF-α level with a significant negative correlation versus serum irisin level in HFD obese rats that represents other mechanism for the pro-
thrombotic tendency observed in our study. This is in accordance with results of Mihara et al. [64].

Obesity is characterized by a chronic low-grade inflammation. Increased lipolysis in obese adipose tissues leads to the release of free fatty acids that stimulate nuclear factor kappa-beta (NF-κB signaling cascades in adipose tissue macrophages [68], which appear to play a significant role in the development of insulin resistance [2].

Consequently, the inflammatory processes stimulate plasminogen activator inhibitor-1 (PAI-1) expression in adipose tissue followed by increase of PAI-1 plasma levels [66]. The alteration of the fibrinolytic system caused by increased circulating levels of PAI-1 has a consistent role in the prothrombotic risk related to obesity [67].

In this study, exercise intervention significantly decreased serum TNF-α with a significant negative correlation versus serum irisin levels in HFD with exercise intervention in comparison with that of HFD group. The protective effect of exercise may be to some extent via its anti-inflammatory effects and/or specific effects on muscle derived peptides, so-called “myokines” [68], including irisin [69].

Furthermore, close links exist also between metabolic syndrome and Oxidative Stress (OS) due to an imbalance between pro-oxidant and antioxidant species in favor of oxidized entities [70]. Indeed, patients with metabolic syndrome exhibit damage due to OS [71]. Non-Estered Fatty Acids (NEFAs), hyperglycemia, TNFα, and oxidized LDL lead to generation of reactive oxygen species in the endothelial cells [72]. This leads to accumulation of the end products of lipid peroxidation such as Malondialdehyde (MDA) and carbonylated proteins [73] in the plasma and blood vessels [74] and predispose atherosclerosis [3].

Our results also showed significantly higher levels of MDA in obese rats, while exercise training produced a significant reduction in this marker, which was correlated negatively with irisin levels in both groups.

Hopkins [75], found that irisin inhibited oxidized-LDL-induced intracellular reactive oxygen species generation and the expression of pro-inflammatory molecules including monocytes chemoattractant protein-1, IL-6, intercellular adhesion molecule-1 (ICAM-1), and vascular adhesion molecule-1 (VCAM-1). Thus irisin can play an anti-inflammatory role [76]. In addition, recent studies showed that irisin suppresses oxidized-LDL and hyperglycemia induced endothelial apoptosis in human umbilical vein endothelial cell via suppressing Bax and promoting the Bcl-2 expression and reducing the caspase-3 expression [76-78].

Taking the present findings together, it can be concluded that: Elevated irisin levels due to chronic exercise training may play a role in improving the metabolic and hemostatic abnormalities associated with obesity in rats. This effect could be mediated via insulin sensitizing, hypolipidemic, anti-inflammatory and antioxidant effects.

Additional studies are required to detect the precise mechanism of irisin action in case of obesity related metabolic disorders.

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Effect of Exercise on the Interplay between Serum Irisin & Some Metabolic & Hemostatic Parameters

تأثیر التمارین الرياضیة علی الکلابع بین مستوی الابریسین
فی مصل الکل وبعض دلائل عمليه الاضی
وآليات فقد النزف في نموذج للسمنة في الجرذان

خلفیة البحث: لقد وجد أن النخل في قابلة الکل لالتخیر بیتم وصلة معرفة بين السمنة وتجلب الدم. الابریسین (Irisin) هو هرمون مكتشف حديثًا يبرز من الخلايا العضلية والأنسجة الدهنية. وهناک تقارير متعددة بشأن مستويات الابریسین وترابیتها مع كلة الجسم ودلالات البداية. كما أن تأثیر التمارین الرياضیة على مستوی هذة الهرمون لا زال مثيرا للجدل.

هدف البحث: صممت هذه الدراسة لتحديد مستوی الابریسین في السمنة الناجمة عن النظام الغذائي العالي الدهن وتحديد تأثیر التمارین الرياضیة الممزگ على تلك المستویات فيما يتعلق ببعض دلائل الاضی وکفاءة الکل وفقد النزف في الجرذان.

مواد وطاقم الدراسة: تم إجراء هذه الدراسة على عدد ثلاثین من ذكور الجرذان البيضاء البالغة الکه بوزنها 179-3173 جم. وقد تم تقسيم الففران بشكل عشوائي علی 3 مجموعات: الأضطراب (50٪) دون ممارسة التمارین رياضیة لمدة 8 أسابيع والجموعة الثانية (80٪) دون ممارسة التمارین رياضیة لمدة 8 أسابيع. والجموعة الثالثة (االسباحة لمدة 90 دقيقة في اليوم لمدة خمسة أيام أسبوعیا) لمدة 8 أسابيع.

في جميع المجموعات تم قیاس مؤشر كلة الجسم ومحیط البطن ومستویات الابریسین والجلوكوز والانسولین مع حساب مؤشر المقاومة للانسولین ومستویات دهن الدم وعامل نزف الدم الفعال والدهن دبی في الکل والغیربوبولیتیجین والکل دابیر في البلازما كما تم قیاس زمن النزف والزنم الکل لجلب الدم وزمن البروتومین وزمن البروتوبیلاستین الجزئی الممزگ.

النتایج: فقد أظهرت الدراسة أن تغذیة الجرذان لمدة 8 أسابيع نتجت بغاز بغاز ما على الکل بناءً على زيادة ذا دلالة إحصاائية في مؤشر كلاة الجسم ومحیط البطن ومستویات كل من الجلوكوز والانسولین ومؤشر اقترب من آثار الگلوکوز والکل وذراع الدم الکل ونکاتة وضع الکل منخفض. قیاس الكثافة بعد تناول الکل دبی في الکل والفصلیتیجین والکل دابیر في البلازما وعلاوة على ذلك، كان هناك انخفاضاً ذا دلالة إحصایة في مستوى الابریسین في المصل في نفس المجموعة (المجموعة الثانية). مع وجود إرتباط سبیل ذا دلالة إحصایة بين هذا الهرمون وجميع الدلالات المذكورة أعلاه وکلکان هناك إرتباط إيجابی ذا دلالة إحصایة مع المستویات المنخفضة من البروتومین دبی على الکل. وعلاوة على ذلك، كان هناك انخفاضاً ذا دلالة إحصایة في زمن النزف والزنم الکل لجلب الدم وزمن البروتومین وزمن البروتوبیلاستین الجزئی الممزگ مع وجود إرتباط إيجابی ذا دلالة إحصایة مع مستوی الابریسین في الدم.

کما أن التمارین الرياضیة قد أدت إلى زيادة ذا دلالة إحصایة في مستویات الابریسین وتحسين ملحوظ في دلائل عملية الاضی المذكورة أعلاه وکلکان ذات دلالة کثافة واضح 난زف مع الحفاظ على نفس النوع من الإرتباط مقابل مستویات الابریسین في المجموعة الثالثة.

الاستنتاج: الإرتفاع الحاد في مستوى الابریسین نتيجة التمارین الرياضیة الممزگ قد يلعب دوراً في تحقيق التمثیل الغذائي والقيام

المتعلقة بقابلیة الکل للالتخیر بیتم نموذج للسمنة في الجرذان.