A Study on the Impact of Irisin "A Novel Myokine" in Ameliorating Polyneuropathy of Experimentally-Induced Diabetic Rats

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Abstract

Background: Irisin is a new myokine, has been known to its effect on diabetes.

Objectives: This work is designated to evaluate the possible neuroprotective role of irisin in ameliorating DPN in an experimental model of type 2 diabetes mellitus (T2DM) in adult male albino rats.

Patients and Methods: 40 male albino rats of local strain were used, the rats will be randomly divided into 4 equal groups, 10 rats each, control group, irisin group, diabetic group and diabetic-irisin group. Diabetes was induced by feeding rats a high-fat diet (60% kcal fat) for 4 weeks, then they will subjected to a single IP injection of Streptozotocin (STZ) of 40mg/kg in 10ml sodium citrate buffer PH 7.4. rats in irisin group will be injected S.C subcutaneously with irisin 150ul per rat for 4 weeks during which the rats will supplemented with a normal chow diet. Rats in diabetic-irisin group will be treated with SC injection of irisin 150ul per rat for 4 weeks during which the rats will be supplemented with a normal chow diet. At the end of the experiments (after 4 weeks), rats will be subjected to the following measurements: Food intake, body weight, height and Body Mass Index (BMI), waist circumference, hot plate analgesia meter test, perirenal fat pads/body weight ratio, Nerve Conduction Velocity (NCV) of the sciatic nerves. Blood samples were collected for measuring serum glucose and serum insulin and Homeostasis Model Assessment of Insulin Resistance (HOMA-IR), HbA1C, serum Tumor Necrosis Factor alpha (TNF-α), sciatic nerve tissue level of irisin receptors gene expression, Glutathione Peroxidase 1 enzyme (GPX1) and Thio Barbituric Acid Reactive Substances (TBARS).

Results: T2DM resulted in substantial alterations in biochemical variables and the markers of inflammation (increase TNF-α and oxidative stress (rise of TBARS and decrease of GPX-1) in the sciatic nerve tissue. Blood samples were collected for measuring serum glucose and serum insulin and Homeostasis Model Assessment of Insulin Resistance (HOMA-IR), HbA1C, serum Tumor Necrosis Factor alpha (TNF-α), sciatic nerve tissue level of irisin receptors gene expression, Glutathione Peroxidase 1 enzyme (GPX1) and Thio Barbituric Acid Reactive Substances (TBARS).

Conclusion: Irisin improve nerve conduction velocity in diabetic neuropathy associated with T2DM.

Key Words: Irisin – Diabetic neuropathy – Nerve conduction velocity – Rats.

Introduction

ACCORDING to the latest International Diabetes Federation (IDF), the global prevalence of Diabetes Mellitus (DM) was about 425 million people between 20-79 years (8.8% of the global population) which is predicted to rise to 629 million by the year 2045 (13.3% of the global population) and the prevalence is increasing at an alarming rate in both developed and developing countries [1]. Diabetic Polyneuropathy (DPN) is one of the most troublesome complications of DM [2]. It usually affects more than 50% of diabetic patients [3].

DPN is usually manifested clinically by unpleasant abnormal sensations such as numbness and tingling (dysesthesia), exaggerated response to painful stimuli (hyperalgesia), pain in response to stimuli that do not normally provoke pain (allodynia), and eventual loss of sensation (analgiesia) in advanced cases [4].

Irisin is a newly discovered exercise-mediated hormone-like myokine. Irisin signals to adipose tissue to induce energy expenditure in a process referred to as 'browning of white adipose tissue', thus dissipates chemical energy in the form of heat [5].

Recently, irisin has gained a great interest as a potential new therapeutic strategy to combat the obesity and its associated disorders, such as type 2 diabetes mellitus (T2DM) [5]. It was reported that moderate increase in circulating irisin level was found to alleviates insulin resistance [6]. Numerous studies focus on the association of irisin with metabolic diseases particularly Type 2-Diabetes Mellitus (T2DM).
Material and Methods

Experimental animals:

Forty male albino rats of local strain (age 2-3 months, each weighing 160-200gm) will be used in this study. The rats will be housed, five per a cage (10 rats per a cage with dimensions 80 X 40 X 30cm), in well-ventilated cages at room temperature under the natural 12 hour day/night cycle with free access to a standard chow diet or High-Fat Diet (HFD) and water ad libitum throughout the whole study period. The rats will be acclimatized for one week prior to the start of the experiments. All experimental procedures were done in Physiology Department, Cairo University, lasted for 8 weeks and were conducted in adherence to the international guide for caring and use of laboratory animals of the National Research Council (NRC) 2002, with the approval of the Ethics Committee of the Menoufia University.

Animal groups:

The rats were randomly divided into 4 equal groups, 10 rats each.

- **Group I (control group):** In this group, the rats were supplemented with a normal chow diet for 4 weeks.
- **Group II (irisin group):** In this group, the rats were injected intraperitoneally (IP) with recombinant irisin (100ng/kg) for 8 weeks during which the rats were supplemented with the normal chow diet [7].
- **Group III (diabetic group):** In this group, the rats were rendered diabetic of type 2 (T2DM) by a single IP injection of Streptozotocin (STZ) of 35mg/kg Body Weight (BW) followed by their supplementation with a High-Fat Diet (HFD) for 8 weeks [8].
- **Group IV (diabetic/irisin group):** In this group, the rats were rendered diabetic as in Group III, then they were injected IP with irisin (100ng/kg) for 8 weeks during which the rats were supplemented with a normal chow diet.

Drugs and chemicals:

Streptozotocin (STZ) (Sigma, Chemical Company, St. Louis, USA), Kits for estimation of serum glucose (Boehringer Mannheim, Germany), Kits for estimation of serum insulin (Cayman chemical, USA), Kits for estimation of glycosylated hemoglobin (HbA\(_1c\)) (Riomidi, France), Kits for estimation of Malondialdehyde (MDA) (Cayman chemical, USA), Kits for estimation Glutathione Peroxidase activity (GPx) (Cayman chemical, USA), Kits for estimation of serum Tumor Necrosis Factor (TNF-\(\alpha\)) ELISA kits: (CatalogNo: K0331196) (KOMA BIOTEC., Seoul, Korea).

Experimental procedures:

At the end of the experimental period of 8 weeks, the rats of all groups were subjected to the following measurements:

1. **Anthropometric measurements of the rats:**
   - **A- Body weight (gm):**
     The animals were put individually in a closed plastic container then were weighted by using a sensitive digital scale at the first day (the initial BW) and at the last day (final BW) of the experiment.
   - **B- Body length (cm):**
     Nose to anus length was measured at the start and the end of the experiment using a plastic, non-extensible measuring tape, with an accuracy of 0.1 cm.
     Simply, each rat was placed on its back, the palm of hands, the thumb and fore finger of the left hand grasping the tail firmly near its base, while the thumb and fore finger of the right hand holding the ears and part of the loose skin of the neck. Approximately the same amount of stretching of the body for measurement was used in all cases as one person held the rat throughout the measuring maneuver. Finally, the length of the rats was measured from the tip of the nose (while the neck was extended) to the anus [9,10].
   - **C- Body mass index (BMI, gm/cm\(^2\)):**
     BMI = body weight (gm)/length\(^2\) (cm\(^2\)). The normal range of BMI of adult rats ranges from 0.45 to 0.68gm/cm\(^2\). This index is used as an indicator of overall obesity, where the cutoff value of obesity is BMI of more than 0.68g/cm\(^2\) i.e the rats were considered obese when the BMI was more than 0.68g/cm\(^2\) [10].
   - **D- Waist circumference (cm):**
     The rats were placed in the ventral position and the Waist Circumference (WC) was assessed in the largest zone of the rats’ abdomen using a plastic non-extensible measuring tape [11].
   - **E- Weighing of the visceral (mesenteric), epididymal, and retroperitoneal pad of fats:**
     The rats were anesthetized by intraperitoneal injection of sodium phenobarbitol (40mg/Kg/BW). Then laparotomy was done then the white adipose tissue in the visceral (mesenteric), epididymal, and retroperitoneal fat pads was removed outside the abdomen and was weighted [12].
F- Calculation of the adiposity index: Adiposity index = Sum of visceral, epididymal, and retroperitoneal fat pads/body weight X100 [13].

2- Sampling of blood and separation of the serum: At the end of the experimental period, and following an overnight fasting, blood samples were collected from each rat of all experimental groups. Blood samples were withdrawn from retro-orbital sinuses in non-heparinized 10ml Ependorf tubes. The blood samples were allowed to clot at room temperature before centrifuging at approximately 3000rpm for 15 minutes, and then the serum was separated. The serum was stored at –20°C till the biochemical analysis [14].

3- Dissection of the sciatic nerve of the rats: Each rat was anesthetized by IP injection of sodium Phenobarbital (40mg/kg BW) then the rat was placed in the right lateral position, where a longitudinal skin incision was performed on the back of the thigh in the line between the greater trochanter and the knee. After skin incision and dissection of the muscles, the sciatic nerve was identified, sectioned and the nerve was removed excised outside the rat’s body without any muscles remnants.

4- Measurement of Nerve Conduction Velocity (NCV) of the sciatic nerve:

A- Preparation: Briefly, for determination NCV, about 2cm of the previously left isolated sciatic nerve was cut and mounted in a nerve chamber containing stainless wires and designed for recording an action potential of the nerves.

B- The power lab. device: Photo (1) and its accessory tools, stimulating and recording electrodes Photos (2,3).

C- Setup and calibration of the Power lab: The red and black clips from the stimulating electrodes were connected to two of the metal wires on opposite sides of the nerve bath. The red (positive) BNC connector from the stimulating electrode was connected to the positive (+) output on the power lab then the black (negative) BNC connector from the stimulator electrode was connected to the negative (–) output on the power lab.

D- Mounting of the sciatic nerve Photo (2): The thicker end of the isolated rat sciatic nerve was placed toward the stimulating electrodes (taking in consideration not to grasp the nerve with forceps). A plastic coated paper clip was used to position the nerve. The nerve was blotted on a piece of tissue or filter paper to remove any excess Ringer’s solution. The cover back was placed on the nerve bath.

E- Calculation of NCV: Using a ruler, we measured the distance in millimeters between the ve black cathode of stimulating electrode and the ve black cathode of recording electrode (i.e the distance between the black negative leads of each of the two recording electrodes) then we recorded this value. Channels 1 and 2 that include the Compound Action Potential (CAP) were selected. We opened the zoom window, and used the Marker and Wave form Cursor to determine the time interval for the CAP to travel between the stimulating and recording electrodes. We placed the marker on the stimulus. Next, we placed the waveform cursor over the Compound Action Potential (CAP) peak. We read the time interval (At) in seconds from the Cursor display in the Lab Chart application window i.e the time elapsed between the application of the stimulus until the peak of CAP.
From the distance and the time, we calculate NCV using the following equation:

\[ \text{NCV (m/sec)} = \frac{\text{Distance between electrodes (meter)}}{\text{Time interval (second)}} \]

5- Homogenization of the sciatic nerve of the rats:

The isolated right sciatic nerve was blotted by filter paper and put in a Petri dish containing a phosphate buffered solution (PBS), pH 7.4 containing 0.16mg/ml heparin to remove any red blood cells and clots.

6- Biochemical analysis:

A- Homeostasis Model Assessment of Insulin Resistance (HOMA-IR): HOMA-IR (a good index of insulin resistance) was calculated as the product of fasting serum glucose (mmol/l) and fasting serum insulin (\( \mu \text{IU/l} \)) divided by 22.5.

B- Serum Tumour Necrosis Factor alpha (TNF-\( \alpha \)): Serum TNF-\( \alpha \) concentration in the rats was determined According to the Principle The biosource international Inc. ratTNF-\( \alpha \) kit is a solid phase sandwich Enzyme Linked-Immuno-Sorbert Assay (ELISA).

C- Thiobarbituric Acid Reactive Substances (TBARS) in the sciatic nerve tissue:

Principle (colorimetric method):

TBARS is positively correlated and reflected the level of Malonaldehyde (MDA). The basic principle of TBARS measurement is that in the assessment procedure Thiobarbituric Acid (TBA) reacts with MDA in an acidic medium at temperature 0.95ºC for 30min to form TBARS of pink colour. The absorbance of the resultant pink product can be measured at 532nm.

D- Glutathione peroxidase enzyme (GPX) in the sciatic nerve tissue: (Ultra violet method)

Sample preparation: Sciatic nerve homogenate it is advisable to homogenize the Sciatic nerve tissues in buffer containing a freshly added reducing agent to maintain GPx enzyme activity. For homogenization buffers, it is recommended that mercapto ethanol or dithiothreitol be added at a final concentration of 1mM.

Statistics analysis:

The SPSS Version 23, (Armnok, NY: IBM Corp) was used for analysis of data. The results were expressed as mean \( \pm \) Standard Deviation (SD). The significance of differences between groups was determined by one-way Analysis of Variance (ANOVA) and post-hoc Tukey test was done. Chi-square test was used to study association between qualitative variables. \( p \)-values <0.05 were considered statistically significant.

Results

The present study showed that, there was a significant increase of the mean NCV (\( p=0.02 \)) in the irisin group compared with the corresponding mean value of the control group. While, there was a statistical significance decrease of the mean NCV in the diabetic group compared with the corresponding value in the control and irisin groups (\( p=<0.001 \) and \( p=<0.001 \), respectively). Interestingly, the mean NCV in the diabetic/irisin group was statistical higher compared with both the control and irisin groups (\( p=0.001 \) and \( p=0.001 \), respectively). Also, the mean NCV in the diabetic/irisin was statistically higher compared with the corresponding value in the diabetic group (\( p=<0.001 \)) [Table 1, (Fig. 1A)].

Also this study showed that statistically, there was no significant difference of BMI (\( p=0.09 \)) in the irisin group compared with the corresponding mean value of the control group. While, there was a statistical significance increase of the mean BMI in the diabetic group compared with the corresponding value in the control and irisin groups (\( p=<0.001 \) and \( p=<0.001 \), respectively). Notably, there was a statistical significance decrease of the mean BMI in the diabetic/irisin group compared with the corresponding value in the diabetic group (\( p=<0.001 \)). However, the value of the mean BMI was still higher in the diabetic/irisin group compared with both the control and irisin groups (\( p=0.001 \) and \( p=0.001 \), respectively) [Table 1, (Fig. 2A)].

Statistically, there was no significant difference of the mean HOMA-IR (\( p=0.99 \)) between the irisin and the control groups. While, there was a statistical significance increase of the mean HOMA-IR in the diabetic group compared with the corresponding value in the control and irisin groups (\( p=<0.001 \) and \( p=0.001 \), respectively). In the diabetic/irisin group, the mean value of HOMA-IR was statistically higher compared with both the control and irisin groups (\( p=<0.001 \) and \( p=<0.001 \), respectively). However, there was a statistical significance decrease of the mean HOMA-IR in the diabetic/irisin group compared with the corresponding value in the diabetic group (\( p=<0.001 \)) [Table 1, (Fig. 1B)].

The mean serum TNF-\( \alpha \) of the control, irisin, diabetic and diabetic/irisin groups were 17.54±4.24, 18.68±4.93, 103.58±9.64 and 41.29±8.55pg/ml, respectively. Statistically, there was no significant difference of the mean serum TNF-\( \alpha \) (\( p=<0.001 \))
between the irisin and the control groups. While, there was a statistical significance increase of the mean serum TNF-α in the diabetic group compared with the corresponding value in the control and irisin groups (\( p < 0.001 \) and \( p < 0.001 \), respectively). The mean serum TNF-α in the diabetic/irisin group was statistically higher compared with both values of the control and irisin groups (\( p < 0.001 \) and \( p < 0.001 \), respectively) [Table (2), (Fig. 2B)].

Clearly, there was a statistical significance increase of the mean serum TNF-α in the diabetic/irisin group compared with the corresponding value in the diabetic group (\( p < 0.001 \)).

Statistically, there was no significant difference of the mean sciatic tissue TBARS (\( p = 0.94 \)) between the irisin and the control groups. While, there was a statistical significance increase of sciatic tissue TBARS in the diabetic group compared with the corresponding value in the control and irisin groups (\( p < 0.001 \) and \( p < 0.001 \), respectively). The mean value of the sciatic tissue TBARS in the diabetic/irisin group was statistically higher compared with both the control and irisin groups (\( p < 0.001 \) and \( p < 0.001 \), respectively).

However, there was a statistical significance decrease of the sciatic tissue TBARS in the diabetic/irisin group compared with the corresponding value in the diabetic group (\( p < 0.001 \)) [Table (2), (Fig. 1D)].

Statistically, there was a significant increase of the mean sciatic tissue GPX (\( p < 0.001 \)) in the irisin group compared with the corresponding mean value of the control group. While, there was a statistical significance decrease of the mean sciatic tissue GPX in the diabetic group compared with the corresponding value in the control and irisin groups (\( p < 0.001 \) and \( p < 0.001 \), respectively). The mean value of the sciatic tissue GPX in the diabetic/irisin group was statistically lower compared with both the control and irisin groups (\( p < 0.001 \) and \( p < 0.001 \), respectively). Notably, there was a statistical significance increase of the sciatic tissue GPX in the diabetic/irisin group compared with the corresponding value in the diabetic group (\( p < 0.001 \)) [Table (2), (Fig. 1C)].

**Fig. (1):** Results are expressed as means ± SD (n=10). Error bars represent standard deviation. Significance was considered when \( p \)-value was \(< 0.05 \).

The marks * and # on top of the columns indicate that values are significantly different, when compared with the corresponding values of control and diabetic group respectively.
A Study on the Impact of Irisin “A Novel Myokine” in Ameliorating Polyneuropathy

Fig. (2): Results are expressed as means ± SD (n=10). Error bars represent standard deviation. Significance was considered when p-value was <0.05.

The marks * and # on top of the columns indicate that values are significantly different, when compared with the corresponding values of control and diabetic group respectively.

Table (1): Nerve Conduction Velocity (NCV), Body Mass Index (BMI), Homeostasis Model Assessment of Insulin Resistance (HOMA-IR).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Irisin</th>
<th>Diabetic</th>
<th>Diabetic/irisin</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCV m/sev</td>
<td>0.10±0.01</td>
<td>0.16±0.04</td>
<td>0.02±0.01</td>
<td>0.33±0.09</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.02*</td>
<td>p&lt;0.001*</td>
<td>p&lt;0.001*</td>
<td></td>
</tr>
<tr>
<td>BMI g/cm²</td>
<td>0.50±0.06</td>
<td>0.46±0.04</td>
<td>0.83±0.07</td>
<td>0.66±0.04</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.01*</td>
<td>p&lt;0.001*</td>
<td>p&lt;0.001*</td>
<td></td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.91±0.35</td>
<td>1.85±0.26</td>
<td>14.32±1.22</td>
<td>5.39±0.79</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.01*</td>
<td>p&lt;0.001*</td>
<td>p&lt;0.001*</td>
<td></td>
</tr>
</tbody>
</table>

Table (2): Tumor Necrosis Factor-alpha (TNF-α), Thiobarbituric Acid Reactive Substances (TBARS), Glutathione Peroxidase (GPx).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Irisin</th>
<th>Diabetic</th>
<th>Diabetic/irisin</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α pg/ml</td>
<td>17.54±4.24</td>
<td>18.68±4.93</td>
<td>103±9.64</td>
<td>41.29±8.55</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.001*</td>
<td>p&lt;0.001*</td>
<td>p&lt;0.001*</td>
<td></td>
</tr>
<tr>
<td>TBARS nmol/gm tissue</td>
<td>4.75±1.30</td>
<td>4.24±1.30</td>
<td>60.31±9.00</td>
<td>20.86±2.59</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.01*</td>
<td>p&lt;0.001*</td>
<td>p&lt;0.001*</td>
<td></td>
</tr>
<tr>
<td>GPx μ/gm tissue</td>
<td>60.24±6.36</td>
<td>72.79±5.46</td>
<td>21.05±3.79</td>
<td>45.27±3.68</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.001*</td>
<td>p&lt;0.001*</td>
<td>p&lt;0.001*</td>
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</tbody>
</table>

Discussion

The current study was designed to evaluate the possible modulating effect of irisin on the nerve conduction velocity, body mass index, HOMA-IR, TNF alpha, TBARS and GPX in experimentally induced type 2DM in adult male albino rats.

Diabetic group:

In the present work it was noticed that in this group, the rats were rendered diabetic of type 2 (T2DM) by a single IP injection of streptozotocin (STZ) of 35mg/kg Body Weight (BW) followed by their supplementation with a High-Fat Diet (HFD) for 8 weeks [8]. And this resulted in a statistical significance decrease of the mean NCV in the diabetic group compared with the corresponding value in the control and irisin groups. This result was in agreement with (Wilson and Wright, 2014) who state that different models of diabetic rats have significant decrease of NCV.

Fahim and colleagues conducted a short-term 2 week study in mice that received a low dose of STZ [18]. These diabetic mice exhibited reductions in both resting membrane potentials and miniature end plate potentials. Further analysis of the neuromuscular junction revealed fewer synaptic vesicles at the nerve terminal, which could contribute to the early MNCV slowing that is often documented [18]. Long-term studies in diabetic mice suggest that neuromuscular junctions become denervated in diabetic mice and are associated with motor dysfunction. These anatomical and behavioral changes were positively affected by insulin delivery [16].
Also, our study showed that there was a statistical significance increase of the mean BMI in the diabetic group compared with the corresponding value in the control and irisin groups, and these results were in agreement with [17].

Hu et al., [18] stated that excess weight and obesity to be a major contributing factor to type 2 DM and its complications for both men and women. Both men and women in the overweight category (25 ≤ BMI ≤ 29.99) were at an increased risk of developing DM, with 30% and 10% greater risks respectively. At 30 ≤ BMI ≤ 39.99 both genders were at a 100% greater risk of DM than counterparts with a normal BMI. BMI ≥ 40 increases the odds of developing DM by as much as 150% for women and 180% for men. These results suggest a stronger association between BMI and onset of DM than was previously documented in similar studies.

The irisin group: It has been noticed in this study that there was a significant increase of the mean NCV in the irisin group compared with the corresponding mean value of the control group.

Supporting the role of FNDC5/irisin in the nervous system, it should be noted another study where it is demonstrated that FNDC5 is required for the adequate neural differentiation of mouse embryonic stem cells (mESCs) [19]. The authors observed that both Fndc5 knockdowns in mESCs during their differentiation after postneuronal progenitor formation and the neuronal differentiation were reduced. Also, Moon et al., (2013) [20] showed that hippocampal neurogenesis is regulated by irisin in a dose-dependent manner. So, while physiological concentrations of irisin (5-10nmol/L) had no effect on mouse H19-7 hippocampal neuronal cells proliferation, pharmacological concentrations (50-100nmol/L) increased proliferation when they were compared to control. This increase seems to occur through signal transducer and activator of transcription (STAT) 3 but not AMPK and/or extracellular signal-regulated kinase (ERK) signalling pathways. This study also shows that there was significant decrease of sum of fat (visceral, epididymal, and retroperitoneal fat) in the irisin group compared with the corresponding mean value of the control group. These results were in agreement with (Pardo et al., 2014) [21].

It has been reported that irisin induces browning of subcutaneous white adipocytes, and enhances energy expenditure and fat oxidation. Irisin is therefore identified as a novel target for prevention and treatment of obesity [22]. The recent discovery of the PGC1-α-dependent myokine FNDC5/irisin, which mediates brown-fat development and thermogenesis in white fat, has opened a new field of research [6,23,24].

The soluble form of FNDC5 binds to unidentified receptors on the surface of WAT to induce the expression of Ucp 1 and other BAT-associated genes partly via increased PPAR-α expression. As a result, irisin may act as a muscle-derived energy-expenditure signal that directly communicates with adipose tissue and induces browning. This effect may improve the WAT metabolic profile and enhance whole-body energy expenditure, making irisin a potential new target for the treatment of metabolic diseases [21].

Diabetic/irisin group: This study had revealed that administration of irisin (100ng/kg) caused a significant improvement in NCV in the diabetic/irisin group when compared to the corresponding value in the diabetic group. Fndc5 is highly expressed in the brain, including the Purkinje cells of the cerebellum [25-27] irisin, the shed form of FNDC5 was identified in human cerebrospinal fluid [28]. In addition, immunoreactivity against the extracellular domain of FNDC5/irisin was detected in human hypothalamic sections, especially paraventricular neurons [28]. Other tissues with high FNDC5 levels include skeletal muscle and the heart. Fndc5 gene expression increases during differentiation of rat pheochromocytoma-derived PC12 cells into neuron-like cells [29].

FNDC5 levels are enhanced after differentiation of human embryonic stem cell-derived neural cells into neurons [30] as well as during the maturation of primary cortical neurons in culture and during brain development in vivo [31].

Knockdown of FNDC5 in neuronal precursors impaired their development into mature neurons (and astrocyte), suggesting a developmental role of FNDC5 in neurons [19]. On the other hand, forced expression of FNDC5 during neuronal precursor formation from mouse embryonic stem cells increased mature neuronal markers (Map2, β-tubulin III and Neurocan) and astrocyte marker (GFAP) and BDNF. However, overexpression of FNDC5 in undifferentiated mouse embryonic stem cells did not have these effects, indicating that FNDC5 supports neural differentiation rather than lineage commitment [32].

Pharmacological doses of recombinant irisin increased cell proliferation in the mouse H19-7 hippocampal cell line [20] furthermore, forced expression of FNDC5 in primary cortical neurons
increased cell survival in culture, whereas knockdown of FNDC5 had the opposite effect [31].

This study has shown that there was a statistical significance decrease of the mean serum glucose in the diabetic/irisin group compared with the corresponding value in the diabetic group. Irisin is believed to act through the stimulation of glucose transporter expression of adipocyte mitochondrial biogenesis and the formation of a phenotype, with the activation of brown adipose tissue thermogenesis and lipid consumption. Under these conditions, glycolysis and oxidative phosphorylation increase energy consumption and improve glucose and lipid metabolism [33].

It had been also noticed that there was a statistical significance decrease of the sciatric tissue TBARS, GPX and TNF-α in the diabetic/irisin group compared with the corresponding value in the diabetic group. Similar results were reported by Zhu et al., (2015) [34] who found that in T2DM, irisin improves endothelial dysfunction via decreasing oxidative stress. These data suggested that irisin might have an antioxidant effect. Protective effects of irisin on endothelia was probably due to suppressing HG/HF-induced activation of PKC-β/iNOS pathways [34]. It has been reported that irisin mediates the beneficial effect of exercise on adipose tissues and brain [6,31].

This study also shows there was a statistical significance decrease of the mean HOMA-IR in the diabetic/irisin group compared with the corresponding value in the diabetic group.

These results were in consistent with Jameel et al., (2015) [35] who state that there is a significant inverse relationship was found between plasma irisin levels and HOMA-IR. It was reported that expression of the exercise-and PGC1-α-induced irisin drives brown fat-like development of white fat and protects diet-induced obesity and diabetes in mouse models [6]. They found that irisin is released into blood from skeletal muscle after proteolysis of the membrane protein FNDC5, stimulates Uncoupling Protein 1 (UCP1) expression, increases total energy expenditure, and then improves glucose tolerance and reduces fasting insulin in animal models [6].

However, there is no evidence available on the pathways about the relationships among serum irisin, adiposity, glucose and insulin levels with insulin resistance in humans, therefore studies are warranted to explore the potential pathways among them, which may be helpful in elucidating the mechanisms of insulin resistance [36]. This study shows that there was a statistical significance increase of the mean sciatic nerve irisin receptor gene expression in the diabetic/irisin group compared with the corresponding value in the diabetic group.

Interestingly, the presence of newly diagnosed, yet untreated T2DM was not associated with a further increase in Fndc5, but levels of expression were comparable to those found in healthy obese individuals. In addition, diabetes led to a 40% decrease in circulating irisin. This is in accordance with the previous recent reports indicating that circulating irisin is significantly lower in patients with T2DM [37,38]. In contrast to muscle, adipose tissue Fndc5 mRNA was decreased in prediabetic and diabetic individuals but not affected by obesity. The only available report in this respect showed reduced adipose tissue Fndc5 mRNA in morbid obesity and T2D [38].

Notably, in T2DM, opposite effects on Fndc5/irisin were observed in vivo and in vitro. Fndc5 expression in diabetic muscle in vivo was not different from healthy obese muscle, and irisin in circulation was significantly reduced in individuals with T2D. In contrast, myotubes derived from patients with T2D expressed the most Fndc5 and produced the most irisin into the media [39].

This has been reproduced in several independent in vitro experiments with almost identical results. The only published information on Fndc5 expression in human primary muscle cells shows a similar positive association between myotube Fndc5 mRNA and fasting insulin and insulin sensitivity (HOMA-IR) in 37 young healthy individuals and 14 elderly men [40]. It is therefore possible that glucose or lipids could represent/trigger the missing regulatory factor associated with lower irisin levels in T2D. Our in vitro experiments clearly support this notion by showing a 40 and 20% reduction in Fndc5 gene expression in muscle cells treated with palmitate and glucose, respectively [39].

Conclusion:
Irisin has been shown to be very efficacious in ameliorating the diabetic polyneuropathy in T2DM through its ability in improving glucose homeostasis and insulin resistance and up regulating UCP-1 expression (a regulator of thermogenic capability of brown fat).

References


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A Study on the Impact of Irisin "A Novel Myokine" in Ameliorating Polyneuropathy


دور الأسيروسين (الهرمون العضلي الجديد) في تحسين إتئاب الأعصاب الطرفية في مرض البول السكري المحدث تجريبياً في الجرذان

مقدمة: وفقاً لآخر تقدمات الاتحاد الدولي لمرض السكري، قد عدد المصابين بداء السكري في جميع أنحاء العالم ينحو 425 مليون مصاب، أو 8.8% من البالغين الذين تراوحت أعمارهم بين 20 و 79 سنة في عام 2017. يعد إتئاب الأعصاب الطرفية الناتج عن داء السكري إحدى أكثر المضاعفات إزعاجاً. يشير إتئاب الأعصاب الطرفية الناتج عن داء السكري بتغيرات وظيفية ويتأثر في الأعصاب الطرفية الحساسة والحركية ويعتبر أكثر من الأطراف البعيدة، ويعتبر أيضاً الأعراض العصبية.

مواد وطريق البحث: تم تنفيذ هذا البحث في قسم علم وظائف الأعضاء، كلية طب جامعة القاهرة، وأجري هذا البحث على 60 جرذ أبيض من الذكور البالغين بالسلالة المحلية، وكانت أوزانه تتراوح بين 160-200 جرام. تم تقييمهم بالتصاوير أربعة مجموعات تحتوي كل منها 10 جرذان. مجموعة ضاربة والمجموعة الثالثة: تم حقن كل جرذ بـ 150 ميكروجرام من الأسيروسين تحت الجلد لمدة أربع أسابيع والجموعة الثالثة: تم حقن كل جرذ بـ 150 ميكروجرام من عقار الاستريوتروسين داخل الفم البريتوتي، والمجموعة الرابعة تم إعطاء دواء عالي الدهن لمدة أربع أسابيع، ثم حقنهم بجرعة واحدة من عقار الاستريوتروسين داخل الفم البريتوتي، وفي نهاية التجربة (بعد 3 أسابيع) خضع الجرذان للقياسات التالية: الطعم المقدم وزن الجسم والعمل ككلة الجسم، وجمال الخصر، وأخيراً قياس تسخين الألم، والضغط الساخن، ونسبة الدهون الحشوية المحيدة بالكيتومي، إلى وزن الجسم، وسرعة التوسيع العصبي الخاصة بالصبب الوركي، ونسبة الجلوكتوزي المصل، نسبة الأمينوس في المصل، نسبة الهيمولوبين المصل، مستوى الميلادغيد داخل نسيج العصب الوركي، مستوى الجلوكتوزي، ورويكسين داخل نسيج العصب الوركي، مستوى الجلوكتوزي، ورويكسين داخل نسيج العصب الوركي.

النتائج: المجموعة الأولى والثانية: لوحظ عدم وجود أي تغير نو دالة إصبعائية في أي من القياسات بين المجموعتين الأولى والثانية. المجموعة الثالثة: تغيرت جميع القياسات الخاصة بهذه المجموعة المصابية بمرض البول السكري بعد حقنهم عباده الاستريوتروسين حيث لوحظ إرتفاع في (نسبة الهيمولوبين المصل، نسبة الأمينوس في المصل، نسبة الدهون الحشوية المحيدة) كما لوحظ انخفاض في (مستوي الإنسولين، مستوي الجلوكتوزي، ويوبريكسيدين، سرعة التوسيع العصبي للصبب الوركي)، وفقاً لهذه النتائج، جاءت هذه المجموعة بالمقارنة بالمجموعة الضارة، المجموعة الرابعة: لوحظ انخفاض في هذه المجموعة المصابية بداء السكري وتحسن الأسيروسين وفقاً لهذه النتائج، وتحسن الملاحظات الجزيئية، تم تحصين الأسيروسين ويوبريكسيدين، وفقاً لهذه النتائج، وتحسن الملاحظات الجزيئية، وتحسن الأسيروسين ويوبريكسيدين.

الاستنتاجات: في ضوء نتائج هذا البحث يمكن الاستنتاج أن الأسيروسين يمكن أن يحسن من إتئاب الأعصاب الطرفية الناتج عن داء السكري من النوع الثاني.