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The Effect of n-3 Polyunsaturated Fatty Acids Supplementation on Serum Irisin in Patients with Type 2 Diabetes: A Randomized, Double-Blind, Placebo-Controlled Trial

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Abstract

Background: Diabetes refers to a group of metabolic diseases with blood glucose of higher than normal ranges. Furthermore, n-3 polyunsaturated fatty acids are necessary for the regulation of the activity of human function. The effect of n-3 PUFA on diabetes has been investigated in animal studies, yet, the exact amount has not been set, to date. Irisin, as a new myokine, is released from skeletal muscle and Irisin levels decrease as a result of physical inactivity, overweightness, and obesity. Also, the reduction of serum irisin level is associated with development of insulin resistance and type 2 diabetes. This study was performed to assess the effects of n-3 PUFA supplementation on serum irisin level in patients with diabetes.

Methods: This randomized clinical trial included 43 patients with type 2 diabetes (21 patients in the placebo group and 22 patients in the n-3 PUFA supplement group). They were randomized to groups, one receiving 10 weeks of either n-3 PUFA supplement and the other the placebo (1250 mg capsule, three times per day). Samples were also matched by age, gender, and body mass index (BMI) in the 2 groups. Anthropometric measurements, demographic information and dietary intakes were obtained both before and after the intervention. Serum irisin levels were measured before and after the intervention using human irisin enzyme linked immunosorbent assay (ELISA) kit. Independent t-test was used to compare the mean outcomes between groups.

Results: At baseline, irisin serum levels were not significantly different between the placebo and n-3 PUFA supplementation groups ($P > 0.05$). However, a significant change was observed between the groups after intervention ($P = 0.04$). Also there was a significant difference in mean change (after versus before the intervention) ($P = 0.05$). Compared to the placebo, n-3 PUFA supplementation decreased serum FBS and HbA1C ($P = 0.036$ and 0.001; respectively). Also, there were significant differences between changes of diastolic blood pressure and HOMA-IR after the intervention between the groups. The duration of illness was not considered as a confounding factor because there was no significant association between irisin level (after versus before the intervention) and the illness duration.

Conclusions: The current study indicated that n-3 PUFA supplementation with a dosage of 1250 mg three times per day, resulted in increased serum irisin level of diabetic patients.

Keywords: Irisin, Type 2 Diabetes, Myokine, n-3 Poly Unsaturated Fatty Acids, Eicosapentaenoic Acid (EPA), Docosahexaenoic Acid (DHA)

1. Introduction

Diabetes refers to a group of metabolic diseases in which the blood glucose level is more than normal amounts over a long time period. In this condition, the patient's ability to produce insulin is impaired or the body becomes resistant to insulin, thus, insulin becomes disabled to perform its normal function (1). It has been found that genetic factors, obesity, and physical inactivity have an important role in type 2 diabetes development (2).

N-3 polyunsaturated fatty acids are necessary for regulating the activity of human function, yet, they are not produced within the body (1). In numerous animal studies, the effect of n-3 PUFA on diabetes has been investigated, yet, the exact dosage has not been determined. The effects of n-3 PUFA are expressed through insulin synthesis and prevention of insulin resistance (3, 4).

Myokines are complex of cytokines, small proteins (5 - 20 kDa), and proteoglycan peptides, which are secreted by myocytes in response to muscular contractions. They have

an important role in preventing insulin resistance along with anti-inflammatory properties (5).

A new myokine has recently been described, which is released by skeletal muscles in response to physical activity and as a result of peroxisome Proliferator-activated receptor Gamma Coactivator 1 alpha (PGC1α) activation, Fibronectin type III Domain-Containing Protein 5 (FNDC5), which is the precursor of irisin, is broken in skeletal muscles. Thus, irisin is secreted in blood and activates thermogenic activity in adipose tissue (6, 7). Also, irisin changes white fat into brown fat (8) and evidence suggests that FNDC5 is effective in the expression of thermogenesis in fat cells, and via this mechanism prevents weight gain (9, 10). Several studies reported that irisin serum level has a significant association with Body Mass Index (BMI), muscle, and fat mass (11). Therefore, irisin could increase the energy expenditure, decrease body weight and obesity, and life expectancy increment, reduce insulin resistance, and lead to metabolism modification (6, 12, 13).

Findings from previous studies showed that irisin can lead to weigh loss and glucose homeostasis improvement, and has a role in cholesterol homeostasis (7, 14).

Irisin levels decrease as a result of physical inactivity, overweightness, and obesity. Also, the reduction of serum irisin level is associated with development of insulin resistance and type 2 diabetes (15). Also, the low serum levels of irisin in diabetic patients are due to the decreased expression and activity of PGC-1α (16).

Irisin can prevent the progression of insulin resistance by improving insulin sensitivity, thus it has been considered as a protective factor against diabetes progression (16-18). A significant negative relationship between irisin level and HbA1C, and fasting insulin was observed (6, 14, 19).

2. Objectives

The aim of our study was to assess the effects of supplementation with n-3 PUFA on serum irisin level in patients with type 2 diabetes.

3. Methods

3.1. Subject and Design

The current study was conducted on 43 patients with type 2 diabetes, who were randomly divided to 2 groups. Twenty-two and 21 patients were categorized in supplement and placebo receiving groups, respectively. This study was a 3-month randomized clinical trial and it included 43 patients with type 2 diabetes, who met the inclusion criteria. The inclusion criteria were as follows: age between 30 to 65 years old, BMI in the range of 25 to 35 kg/m^2, and history of at least one year from illness onset.

Samples were collected from diabetes centers of Taban, Shariati hospital and Iran diabetes association. All eligible individuals were randomly divided to 2 groups. Permuted block randomization with blocks of size 4 was used for randomization. The samples were matched by age, gender and BMI in both groups, and 21 subjects were allocated to the placebo group (13 males and 8 females), and 22 subjects were located to the n-3 PUFA supplement group (12 males and 10 females). Although, 44 patients were enrolled at the beginning of the study, one of them was excluded because of failure in consuming supplements.

The exclusion criteria were as follows, having received n-3 PUFA supplements during the past 3 months, having chronic kidney or hepatic diseases, hematologic disorders, microalbuminuria, hypo or hyperthyroidism, and heart disease, gestation and breastfeeding, taking contraceptives or corticosteroids, insulin or thiazolidinediones treatment, and consumption of dietary supplements, vitamins, and herbal products during the last 3 months and via the intervention.

The study was approved by the ethical committee of the diet and nutrition faculty of Tehran University of Medical Sciences, and at the beginning of the study an informed written consent was obtained from all participants. The registration code for this project was IRCT2015061922769N3 on Iran clinical trial site. Also, the ethics code of the project was IR.TUMS.REC.1394.270.

3.2. Anthropometric and Biochemical Measurements

Participants were followed up by weekly phone calls. All participants provided 10 cc fasting venous blood samples in the morning after 12 to 14 hours at baseline and at the completion of 10 weeks of intervention for measurement of several parameters. Body weight was measured to the nearest 0.1 kg using a digital Seca (Seca GmbH & co KG, Hamburg, Germany) scale, with subjects wearing light clothing (i.e. no sweaters, jackets, or belts) and no shoes. Height was measured to the nearest 0.1 of a centimeter using a wall-mounted stadiometer (Seca GmbH & co KG, Hamburg, Germany). A flexible tape was used to assess waist and hip circumferences to the nearest 0.5 centimeters. The waist circumference was assessed at half the distance between the last rib and the iliac crest, and the hip circumference was taken at the largest anterior protrusion.

During each visit, demographic data for each participant was obtained. In addition, a three-day 24-hour food recall was collected by interviews. The recorded data were analyzed by the Nutritionist-4 software. At the end of supplementation (10 weeks), study participants were invited to the laboratory, while fasted for 12 to 14 hours. Then, 10

cc of venous blood sample was taken again and all measurements were re-evaluated. In order to follow up supplements consumption, patients received a phone call and both groups were reminded to take supplements.

Human Irisin ELISA kit (ZellBio GmbH, Germany) was used twice (before and after the intervention) in order to measure serum irisin. The human Irisin assay kit sensitivity was about 0.02 μg/mL. According to the protocol outlined in the irisin kit, the unit was μg/mL.

3.3. Intervention

Participants in supplement group received 3 capsules of n-3 polyunsaturated fatty acids (Zahravi pharmaceutical company, Tabriz, Iran) per day. Each capsule contained 1 250 mg n-3 PUFA, which included 300 mg of DHA and 600 mg of EPA. The rest of the capsule (350 mg) was a filler ingredient. On the other hand, the control group received placebo capsules containing paraffin. Thus, each subject in the first group received 3 capsules of omega-3 fatty acids daily and it was recommended to be taken during breakfast and dinner. The second group received 3 placebo capsules per day, which contained paraffin. These capsules were similar to n-3 PUFA capsules in terms of appearance and they were used with the same method. Moreover, the capsules were the same in terms of appearance, color, size, and odor so that the researcher and patients were not able to detect the placebo and n-3 PUFA. Due to the double-blind design of the study, the principal researcher of the study was unaware of placebo and n-3 PUFA capsules, and the third person packaged and coded the capsules. At the end of the study, codes were delivered to the researcher. The patients were given supplements twice. Some of the supplements were given at baseline and new supplement boxes were given after 5 weeks of intervention (concurrent with the next visit of patients) by delivering the finished supplement box.

3.4. Statistical Analysis

Normality of the data was evaluated by the Kolmogorov-Smirnov test. Therefore, to describe quantitative variables, the mean (SD) or median (interquartile range), and for qualitative variables, frequency (percentage) was reported. Differences between before and after intervention values were tested using a paired t test or wilcoxon exam. To compare the mean of outcomes in quantitative variables between the 2 groups, independent t test was used. Also for comparison of quality factors between groups, chi-square test and Fisher's exact test was applied. The SPSS software (version 21, SPSS Inc, Chicago, IL, USA) was applied for data analysis and statistical significance was accepted at $P < 0.05$.

4. Results

Baseline characteristics of study participants across 2 groups of placebo and intervention are presented in Table 1. A total of 43 participants completed the study with mean (SD) age of 49.88 (6.88) years and approximately 58% were female. As indicated, no significant differences were found in terms of all presented variables between the 2 groups, except for duration of illness.

Table 1. Anthropometric Indices, Biochemical Measurements, Blood Pressure and Dietary Intakes of Study Participants at Baseline (Before the Intervention)[a]

Variable	Placebo Group (N = 21), (M = 13, F = 8)	n-3 PUFA Group (N = 22), (M = 12, F = 10)	P Value[b]
Age, y	51.34 ± 1.36	48.44 ± 1.42	0.148
Weight, kg	79.3 ± 2.0	79.7 ± 2.22	0.896
Height, m	1.64± 0.18	1.67± 0.01	0.376
BMI, kg/m^2	29.1 ± 0.4	28.39 ± 0.46	0.266
WC, cm	104.3 ± 1.6	99.9 ± 1.4	0.051
HC, cm	106.22 ± 1.23	105.4 ± 1.04	0.609
WHR	0.98 ± 0.01	0.94 ± 0.01	0.064
Duration of illness, y	6.54 ± 0.71	4.54 ± 0.55	0.031
FBS, mg/dL	1.78 ± 9.54	1.75 ± 8.53	0.833
Insulin, μm/mL	12.25 ± 0.91	10.41 ± 0.78	0.153
HbA1C %	7.72 ± 0.22	7.68 ± 0.25	0.902
HOMA.IR %	5.30 ± 0.39	4.57 ± 0.48	0.245
Serum Irisin, μg/mL	1.69 ± 0.20	2.12 ± 0.21	0.152
Systolic BP, mmHg	131.7 ± 2.2	127.2 ± 1.9	0.13
Diastolic BP, mmHg	85.20 ± 1.99	81.66 ± 1.64	0.17
Total energy, kcal	2089 ± 53	2081 ± 43	0.9
Total protein, g	67.74 ± 4.81	72.41 ± 4.78	0.49
Total fat, g	88.59 ±4.65	77.74 ± 3.59	0.06
Total carbohydrate, g	262 ± 7	278 ± 9	0.17
DHA, g	0.05 ± 0.03	0.15 ± 0.09	0.31
EPA, g	0.017 ± 0.01	0.05 ± 0.02	0.31

Abbreviations: BMI, body mass index; BP, blood pressure; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; F, female; HC, hip circumference; M, male; WC, waist circumference; WHR, waist-to-hip ratio.
[a]Values are described as mean ± standard error (SE).
[b]Independent sample t-test.

Table 2 indicates changes of studied variables during the intervention. Significant changes of HOMA.IR, serum irisin level, and diastolic blood pressure were obtained after 10 weeks of intervention (P = 0.04 for all). Also, levels of fetal bovine serum (FBS) (P = 0.036) and hemoglobin A1C

(HbA1C) (P = 0.001) decreased significantly after the intervention in the n-3 PUFA supplement group. Mean serum irisin level was 1.69 ± 0.95 μg/mL in the placebo group and 2.12 ± 0.98 μg/mL in the supplement group, at the beginning of the study. Although, after the intervention it increased to 1.72 ± 1.61 μg/mL and 3.61 ± 3.84 μg/mL, respectively. According to t test results, no significant difference was seen before the intervention (P = 0.15). No significant difference was observed in serum irisin level before and after the intervention in the placebo group (P = 0.888), yet, the difference was significant in the supplement group during the intervention (P = 0.045). There were significant changes in means of serum irisin levels (after versus before intervention) across the 2 study groups during the intervention (1.48 ± 0.69 vs 0.02 ± 0.02 kg/ml, P = 0.05).

Table 2. Anthropometric Indices, Biochemical Measurements, Blood Pressure and Dietary Intake of Study Participants (After Intervention)[a]

Variable	Placebo Group (N = 21), (M = 13, F = 8)	n-3 PUFA Group (N = 22), (M = 12, F = 10)	P Value[b]
Weight, kg	79.26 ± 2.07	79.44 ± 2.31	0.956
BMI, kg/m^2	29.08 ± 0.45	28.28 ± 0.47	0.232
WC, cm	103.96 ± 1.73	99.87 ± 1.47	0.741
HC, cm	105.9 ± 5.8	105.4 ± 1.0	0.609
WHR	0.98 ± 0.01	0.94 ± 0.01	0.064
FBS, mg/dL	1.70 ± 9.10	1.57 ± 9.88	0.327
Insulin, μm/mL	12.52 ± 0.90	10.22 ± 0.88	0.076
HbA1C, %	7.64 ± 0.20	7.10 ± 0.22	0.80
HOMA.IR, %	5.62 ± 0.55	4.09 ± 0.50	0.047
Serum Irisin, μg/mL	1.72 ± 0.35	3.61 ± 0.82	0.043
Systolic BP, mmHg	130.2 ± 2.2	127.1 ± 1.7	0.27
Diastolic BP, mmHg	86.00 ± 1.86	81.45 ± 1.19	0.04
Total energy, kcal	2091 ± 51	2088 ± 43	0.97
Total protein, g	67.48 ± 3.48	73.03 ± 3.73	0.28
Total fat, g	82.18 ± 3.94	79.88 ± 2.53	0.62
Total carbohydrate, g	276 ± 9	275 ± 7	0.9
DHA, g	0.08 ± 0.04	0.19 ± 0.1	0.34
EPA, g	0.03 ± 0.01	0.06 ± 0.03	0.37

Abbreviations: BMI, body mass index; BP, blood pressure; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; F, female; HC, hip circumference; M, male; WC, waist circumference; WHR, waist-to-hip ratio.
[a] Values are described as mean ± standard error (SE).
[b] Independent sample t-test.

The duration of illness was not considered as a confounding factor in study models, because there was no significant association between the change in irisin level (after versus before the intervention) and the illness duration. Therefore, the obtained results were only related to the intervention with n-3 PUFA supplement and duration of illness not affecting the results. Also, there was no statistically significant correlation between duration of illness and irisin diff (P = 0.586), (Pearson correlation = 0.08).

5. Discussion

To the best of our knowledge, this was one of the first studies assessing the effect of n-3 PUFA supplementation on irisin serum level in type 2 diabetes patients. Also, the current study indicated that omega-3 supplementation with a dosage of 1250 mg three times per day, resulted in an increased serum irisin level of patients with diabetes, and changes in HOMA.IR, serum irisin level, and diastolic blood after the intervention between the 2 groups. Also levels of FBS and HbA1C decreased significantly after intervention in the n-3 PUFA supplement group. However, no significant difference was observed in the case of weight, body mass index, waist circumference, hip circumference, waist-hip ratio (WHR), and systolic blood pressure variables, and there was no significant difference between duration of illness and irisin diff (after versus before intervention).

Reduced serum level of irisin is associated with the development of insulin resistance and type 2 diabetes (20). Some studies have also found that increasing n-3 PUFA can lead to Irisin synthesis or secretion via the activation of PPARγ (6, 7).

Both turning white adipose tissue into brown and thermogenesis through increasing UCP-1 are the main functions of irisin in the body (21). Increment in the number of UCP-1 in brown adipose tissue leads to an increase in fat oxidation, energy expenditure, and insulin sensitivity (22). Irisin is considered as an important protective factor against diet-induced obesity because of increasing energy expenditure due to increased activation of brown adipose tissue. Also, it is an effective treatment strategy to deal with metabolic disease (16, 23).

5.1. Human Studies

Choi et al., performed a cross sectional study on 104 patients, who were recently diagnosed with type 2 diabetes and 104 participants with normal glucose level. They perceived that irisin serum level has a meaningful relationship with BMI, 2-hour postprandial glucose, HbA1C, and triglyceride (TG). Nevertheless, no significant difference was seen between serum Irisin level and WHR, FBS, fasting insulin, lipid profile, and HOMA-IR. Also, they observed a reduction in serum Irisin level in diabetic patients (20).

In another case-control study conducted by Lio et al. in Singapore, both patients with type 2 diabetes and healthy individuals were investigated, and a significant difference was found between diabetic and non diabetic subjects in the case of irisin level after adjusting for age, gender, and BMI. Also, irisin concentration was significantly associated with age, BMI, total cholesterol, FBG, TG, and diastolic blood pressure. However, no significance relationship was observed between serum irisin level by serum adiponectin, hs-CRP, HDL, and systolic blood pressure (12).

The results of a study on human rhabdomyosarcoma cells have shown that treatment with omega-3 fatty acids for 24 and 48 hours leads to increased expression of the irisin (24).

Also, the study of Moreno-Novarrete et al. showed that concentrations of irisin circulation and irisin gene expression in muscle and adipose tissue in patients with type 2 diabetes and obesity is reduced (25).

In another study, which was carried out on 115 subjects with central obesity (waist circumference more than 90 cm for males and more than 80 cm for females), the results showed a significant relationship between male gender and muscle mass with serum irisin level. No significant difference was seen in the amount of systolic and diastolic blood pressure, BMI, body fat percentage, lipid profile, fasting blood glucose, fasting insulin, and HbA1C by irisin level in both groups. Also, reduced serum irisin level did not lead to increased blood pressure, TG and decreased HDL (24).

5.2. Animal Study

Seo et al. studied thirty-three 3-week-old Sprague Dawley rats that were fed by a high fat diet in order to evaluate the effect of garlic extract and endurance exercise on FND5 and circulating irisin level. They observed that aged garlic extract and exercise training intervention have protective effects against insulin resistance caused by high fat diet, yet, this effect was independent of serum irisin and skeletal muscle FNDC5 (15).

5.3. Strengths and Limitations

Of the study strengths we can note random and double blind sampling. Also, this was the first study, which has assessed n-3 PUFA consumption on irisin serum level in patients with diabetes. In addition, the participants were within a specific area of age, gender, and BMI, and these factors were matched. Also, the sample loss was low in the current study. However, there were several limitations in this study. Firstly, we did not measure body composition and physical activity, which are 2 important factors for gene expression and irisin secretion. Other cytokines such as adiponectin, Tumor Necrotising Factor (TNF)-α, Interleukin 6 (IL6), and IL8, which can be linked by irisin serum level were not measured. Also, samples were not studied in terms of diabetes complications, type and amount of drugs, and job type, which can influence serum irisin level. The small sample size was another limitation, especially to assess anthropometric indices.

5.4. Conclusions

In the current study we found that omega-3 supplementation with a dosage of 1800 mg EPA and 900 mg DHA, could significantly increase serum irisin level.

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Footnote

Conflict of Interest: The authors declare that they had no conflict of interest.

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