Original Article

Omega-3 Fatty Acid Could Increase One of Myokines in Male Patients with Coronary Artery Disease: A Randomized, Double-Blind, Placebo-Controlled Trial

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Abstract

Background: Omega-3 fatty acids have a protective role against cardiovascular disease and these protective properties are attributed to its anti-inflammatory effects. Myokines have anti-inflammatory properties and thereby reduce low-grade inflammation. Irisin, as a myokine, is considered to be therapeutic for human metabolic diseases. This study was conducted to determine the effects of Omega-3 fatty acids supplementation on serum irisin in men with coronary artery disease (CAD).

Methods: This study was an 8-week randomized, double-blind, placebo-controlled trial. Forty-eight CAD male patients (Omega-3, n = 24; control, n = 24) were randomly assigned to either Omega-3 or control groups by permuted block randomization method. Only the participants with more than 50% stenosis in at least one major coronary vessel were included. A total of 3 participants in the control group were excluded from the study. Forty-five participants (Omega-3, n = 24; control, n = 21) completed the study. Participants took Omega-3 fatty acids supplement (720 mg eicosapentaenoic acid plus 480 mg docosahexaenoic acid) or placebo (edible paraffin) for 8 weeks. Serum irisin, high-sensitivity C-reactive protein (hs-CRP), lipid profile and anthropometric indices, body composition, and food intake were assessed before and after intervention. Statistical analyses were performed using SPSS software. Paired t-test was used for evaluating within-group effects from baseline. Variables with normal distribution were compared by independent t-test between 2 groups.

Results: Compared to placebo, Omega-3 fatty acids increased serum irisin ($P = 0.044$) and decreased serum hs-CRP ($P = 0.018$) and LDL cholesterol ($P = 0.031$). Omega-3 fatty acids supplementation did not result in any significant changes in anthropometric measurements, blood pressure, serum lipids except for serum LDL, fasting blood glucose, body composition or serum insulin levels (all $P > 0.05$).

Conclusion: Omega-3 fatty acids supplementation could elevate serum irisin in male patients with CAD. Also, these fatty acids may able to decrease serum hs-CRP and LDL cholesterol.

Keywords: Coronary artery disease (CAD), docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), irisin, myokines, omega-3 fatty acids

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Introduction

Coronary artery disease (CAD) has become a public health problem with high morbidity and mortality worldwide, accounting for about 50% of all deaths annually in Iran.[1] In the past decades, many conventional risk factors of CAD have been established, including age, sex and family history, diabetes mellitus, smoking, dyslipidemia, hypertension and obesity,[2] but the exact pathogenesis of CAD is not fully clarified yet.[3] However, growing evidence suggests a causative role for inflammation and oxidative stress in both initiation and progression of CAD.[3,4]

Skeletal muscle has been recently recognized as an endocrine organ expressing a variety of cytokines, termed "myokines".[5] Myokines behave in autocrine, paracrine, or endocrine hormone-like fashion[5,6] and exert endocrine effects on visceral fat, glucose and lipid metabolism.[6] Myokines acting in systemic anti-inflammatory environment could reduce low-grade inflammation and thereby prevent metabolic related diseases.[5,7] To date, the identified myokines comprise interleukin-6 (IL-6), IL-8, IL-15, brain-derived neurotrophic factor (BDNF), leukemia inhibitory factor (LIF),[5] fibroblast growth factor 21, myonectin and myostatin.[8]

Most recently, a novel myokine named "irisin" has been identified; it is regulated by PPARγ coactivator-1alpha (PGC-1a).[9] PGC-1a mediates many biological programs related to glucose, lipid, and energy homeostasis.[10] Furthermore, PGC-1a promotes the expression of fibronectin type III domain containing-5 (FNDC5) gene in skeletal muscle.[11] The FNDC5 gene encodes a type I membrane protein that is proteolytically cleaved and secreted as irisin into the circulation.[12]

In line with these findings, it is supposed that through increasing expressions of uncoupling protein 1 (UCP1) and Cidea, irisin mediates the browning of white adipose tissue.[13] Therefore, irisin could increase energy expenditure by enhanced thermogenesis; this has appealed a lot of attentions regarding potential use of

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irisin as a new treatment preference for obesity and its associated disorders, such as type 2 diabetes mellitus.[13] Based on previous researches, both the diabetic state by itself and its originating metabolic disorders could lead to decreased level of circulating irisin.[12] Hence, reduced irisin level might increase cardiovascular events thorough modulating diabetes mellitus emergence as a risk factor for coronary artery disease.[14] Furthermore, irisin has been shown to be associated positively with HDL cholesterol levels and negatively with triglyceride levels.[13,15] Thus, it is supposed that irisin has a beneficial role in the improvement of blood lipid profile.[13,15]

Dietary supplementation with Omega-3 fatty acids, including docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), is a prevailing habit, and has been ascribed with several health benefits for the cardiovascular system.[16] Several studies have shown that DHA and EPA can exert anti-inflammatory activities through which they could prevent cardiovascular disease.[17] Via another mechanism, Omega-3 fatty acids may exert beneficial effects on prevention of cardiovascular disease by induction of irisin secretion.[18]

Findings from human rhabdomyosarcoma cells under treatment with Omega-3 fatty acids have shown their capability for induction of PGC-1α.[18] Notably, the results of the mentioned study indicated that treatment for 24 and 48 hours with Omega-3 fatty acids could significantly induce irisin expression.[18] We hypothesized that as in the case of physical activity, Omega-3 fatty acids supplementation may also increase irisin levels and thus affect metabolism and improve the risk of metabolic disorders. Since no study has been conducted to evaluate the association of omega-3 fatty acid with serum irisin in CAD, this study was designed to assess the effect of omega-3 fatty acids on serum irisin in men with CAD.

Materials and Methods

Study population

Forty-eight men with CAD aged 45 to 65 years were recruited and 3 participants were lost to follow-up. The participants were recruited between Aug 2012 and May 2013 from the cardiology clinic of Tehran Heart center. The eligibility criteria for this study were more than 50% stenosis documented in at least one major coronary artery in the last 3 months, non-smoking patients with a body mass index (BMI) of ≤ 30 and no infections, allergies, thyroid dysfunction, diabetes mellitus, kidney and liver diseases, cancer or myopathies. Participants were excluded in case of using warfarin, multivitamins, fish oil or Omega-3 fatty acids supplements during the preceding three months. Smokers were not enrolled; smoking was defined as at least 5 cigarettes per day during the last 6 months. All participants provided written informed consent. All participants were asked not to change, as much as possible, their lifestyle, dietary habits and physical activity levels during the intervention. All participants were requested to report any change in medications.

Study design

This study was conducted as a double-blind, randomized, placebo controlled clinical trial. The sample size for the study was calculated based on the Brain-derived neurotrophic factor (BDNF) and with the aim of improving the serum BDNF level, we needed 21 participants to complete the study.[19] After screening, all eligible CAD patients were randomly allocated in a 1:1 ratio to Omega-3 treatment or to matching placebo by permuted block randomization method. The corresponding author generated the allocation sequence and assigned participants. Another person who was not involved in the intervention enrolled participants. All participants and investigators were blinded to treatment allocation. Participants in Omega-3 group received Omega-3 fatty acid supplementation while those in control group took placebos with the same size and color as Omega-3 fatty acid soft gels. Omega-3 fatty acid soft gels (Minoo Pharmaceutical, Cosmetic and Hygienic Co., Tehran, Iran), containing 720 mg EPA and 480 mg DHA were consumed twice per day by the Omega-3 group. The control group received 4 placebo soft gels per day. Placebo soft gels contained edible paraffin prepared by the same manufacturer (Minoo Pharmaceutical, Cosmetic and Hygienic Co., Tehran, Iran). All participants took 2 soft gels with lunch and 2 soft gels with dinner. The intervention lasted 8 weeks. The demographic characteristics of the participants were collected at baseline. This trial was registered at URL: http://www.clinicaltrials.gov under registration number NCTO2382471.

Anthropometric measurements

Anthropometric parameters were measured at the beginning and at the end of the intervention, including weight, height, waist circumference (WC) and hip circumference (HC). Body weight was measured using a digital scale (Seca, Germany) in light clothing and without shoes. Height was measured using a mechanical measuring tape (Seca206, Germany) in standing position without shoes. BMI was calculated as the weight in kilograms divided by squared height in meters. WC and HC were taken with non-stretchable measuring tape in standing position; WC (in cm) was measured in the upright position at midpoint between the lower level of costal margin and the iliac crest and HC was defined as the widest circumference of the hip. Waist to hip ratio (WHR) was calculated as WC in centimeters divided by HC in centimeters. All the measurements were made according to WHO recommendations. Body composition was determined using bioelectrical impedance (Takara BC- 418, Japan).

Biochemical measurements

A blood sample was taken in the morning after 12 – 14 hours from all participants. Sera were separated by centrifugation (3500 rpm for 10 min at 4°C) and stored at -70°C until analysis. Serum irisin level was measured by ELISA method using commercial kit (Cat.No: E3253Hu) according to the manufacturer's instructions. Its sensitivity was 0.023 μg/mL. Serum insulin (DiaMetra, Perugia Italy) and high-sensitivity C-reactive protein (hs- CRP) (Labor Diagnostika Nord (LDN), Germany) concentrations were measured by ELISA kits. Serum glucose concentration was determined using the glucose oxidase method with commercial kit (Pars Azmun, Iran). Serum lipid profiles were measured enzymatically using commercial kits (Pars Azmun, Iran) and auto-analyzer system (Selectra E, Vitalab, Netherland).

Blood pressure

Two morning blood pressure readings were recorded and averaged in seated subjects after a 5-min rest using a digital sphygmomanometer (Zyklusmed, Monheim, Germany) before and after intervention.

Physical activity and dietary intake

The participants' physical activity was classified as low active, moderately active or very active based on a structured interview

and using IPAQ (international physical activity questionnaire) based on their occupation, transportation habits, leisure time activities, and whether they participated in regular exercise. Information about daily energy and macronutrient intakes were obtained by 24-hour food recall method based on an interview by trained nutritionists at baseline and after 8 weeks of intervention.

Statistical analysis

Descriptive statistics are presented as mean ± SE. The baseline measurements and dietary intakes of participants in two groups were compared using independent samples *t*-test. To compare ratio variables in the same groups, paired samples *t*-test was utilized. Normality of data distribution was evaluated by Kolmogorov–Smirnov test. All tests were conducted two-sided, and *P*-value < 0.05 was considered statistically significant. Statistical analysis was performed using SPSS version 21.

Results

The trial profiles are summarized in Figure 1 along with the number of participants who completed the study in each step. A total of 3 participants in the control group were excluded from the study since one participant was on trip and two participants underwent open heart surgery during the intervention. Forty-five participants (24 patients in Omega−3 fatty acids supplement group and 21 patients in control group) completed the study. There was no significant difference between the groups regarding mean age (*P*= 0.150; 55.00 ± 1.29 years in Omega-3 group; 57.76 ± 1.36 years in control group). Moreover, no significant differences were observed in terms of dosage and type of lipid lowering and antihypertensive drugs and physical activity between the two groups at baseline (all $P > 0.05$).

There were no significant difference in some confounders including BMI, smoking, hypertension and hypercholesterolemia, triglyceride (TG), LDL-C, or eating habits in the Omega-3 and control groups at baseline (all $P > 0.05$). Similarly, no significant differences were observed between the two groups with respect to energy intake, macronutrients and micronutrients intakes and intake of Omega-3 fatty acids and Omega-6 fatty acids (all $P > 0.05$). Furthermore, nutrient and energy intakes and usual diet of individuals did not change significantly in any of the groups during the study. At 8 weeks of follow-up, participants who received daily 4 soft gels of Omega-3 fatty acids, at a dose of 720 mg EPA and 480 mg DHA had a significantly increased serum irisin; mean serum irisin increased significantly from 2.08 ± 0.17 µg/mL to 2.66 ± 0.30 µg/mL ($P = 0.044$), while serum irisin decreased non significantly from 2.52 ± 0.48 µg/mL to 1.85 ± 0.13 µg/mL in the control group ($P = 0.118$).

Regarding inflammatory marker, serum hs-CRP concentration decreased from 2.96 ± 0.41 mg/L to 1.86 ± 0.15 mg/L in the Omega-3 group ($P = 0.018$), but no change was observed in the control group at the end of the study period. Also, the results of this study showed that participants who received Omega-3 fatty acids supplements had significantly decreased serum LDL levels ($P = 0.031$). The baseline and mean changes in both anthropometric and body composition characteristics of participants are shown in Table 1. Table 2 shows the baseline and mean changes in blood pressure of participants in the two groups. After 8 weeks of follow-up, there was no change in BMI, blood pressure, serum lipids except for serum LDL concentration, fasting blood glucose, fat mass, fat free mass, waist circumference, hip circumference, pulse rate (data not shown) or serum insulin in the group treated with Omega-3 fatty acids supplementation (all $P > 0.05$). Table 3 and Table 4 respectively display the baseline and mean changes in biochemical characteristics and energy and macronutrients intakes in Omega-3 and control groups.

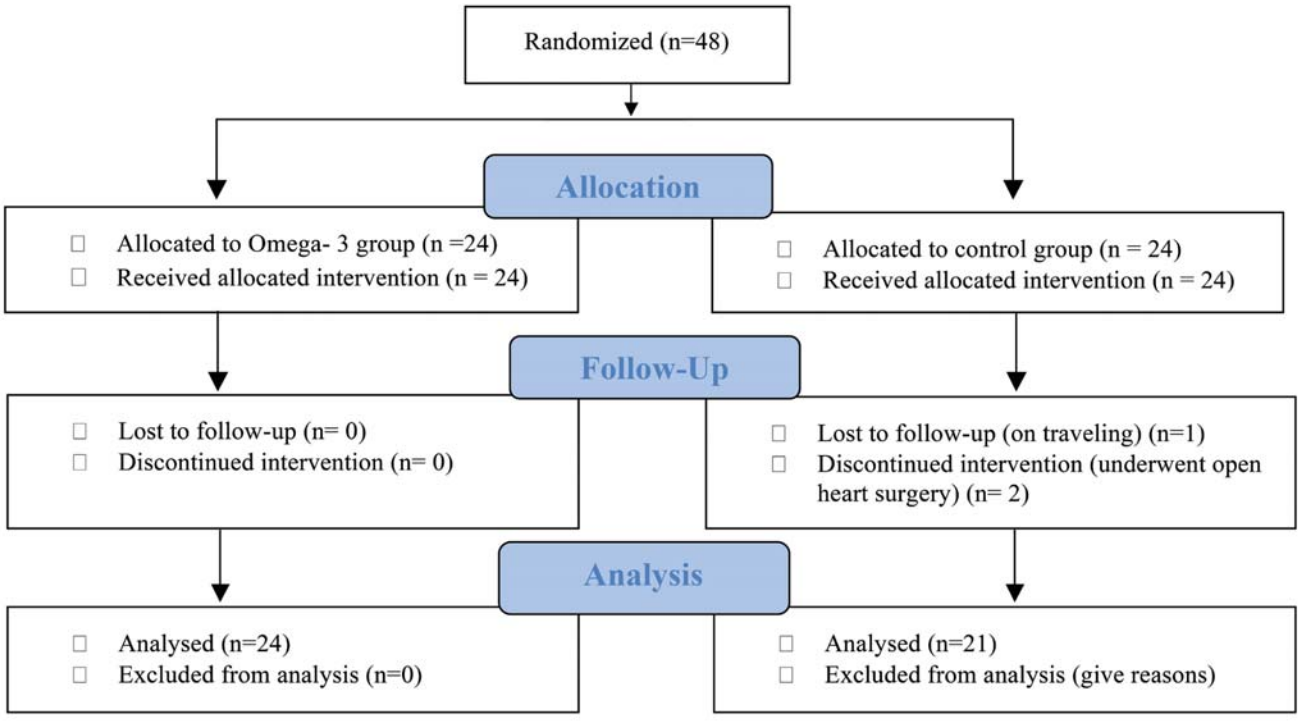

Figure 1. Flow chart of the randomized control trial

Table 1. Anthropometric indices and body composition at baseline and their changes during intervention*

		Omega-3 group (N = 24)	Control group (N = 21)	*P*-value #
Weight (kg)	Baseline	81.08 ± 2.18	77.36 ± 2.06	0.225
	Changes	-0.18 ± 0.31	0.65 ± 0.39	0.104
Body mass index (kg/m²)	Baseline	28.34 ± 0.68	27.53 ± 0.75	0.430
	Changes	-0.07 ± 0.11	0.21 ± 0.13	0.111
Fat Mass (kg)	Baseline	20.66 ± 1.55	18.32 ± 1.37	0.272
	Changes	-0.41 ± 0.57	0.65 ± 0.55	0.203
Fat Mass (%)	Baseline	25.21 ± 1.46	23.34 ± 1.38	0.361
	Changes	-0.45 ± 0.62	0.78 ± 0.67	0.189
Fat Free Mass (kg)	Baseline	59.70 ± 1.31	58.89 ± 1.39	0.675
	Changes	0.72 ± 0.48	0.27 ± 0.43	0.508
Fat Free Mass (%)	Baseline	74.80 ± 1.45	76.66 ± 1.38	0.363
	Changes	0.43 ± 0.62	-0.79 ± 0.68	0.196
Waist circumference (cm)	Baseline	98.93 ± 1.75	97.81 ± 1.75	0.653
	Changes	-0.06 ± 0.43	0.57 ± 0.38	0.288
Hip circumference (cm)	Baseline	102.64 ± 1.21	99.85 ± 1.09	0.100
	Changes	- 0.56 ± 0.47	0.33 ± 0.36	0.154
Waist to hip ratio	Baseline	0.96 ± 0.01	0.97 ± 0.01	0.392
	Changes	0.004 ± 0.004	0.002 ± 0.003	0.756

*The results are described as mean ± Standard Error (SE); # Independent sample *t*-test

Table 2. Blood pressures at baseline and their changes during intervention*

		Omega-3 group (N = 24)	Control group (N = 21)	*P*-value #
SBP (mmHg)	Baseline	122.88 ± 3.10	125.43 ± 3.35	0.579
	Changes	-4.75 ± 2.66	-0.28 ± 2.72	0.250
DBP (mmHg)	Baseline	81.29 ± 2.32	77.86 ± 2.37	0.308
	Changes	-3.70 ± 2.86	0.23 ± 1.21	0.214

*The results are described as mean ± Standard Error (SE); # Independent sample *t*-test; SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure.

Table 3. Serum irisin and biochemical characteristics at baseline and their changes during intervention*

		Omega-3 group (N = 24)	Control group (N = 21)	*P*-value #
Serum irisin (µg/mL)	Baseline	2.08 ± 0 .17	2.52 ± 0.48	0.394
	Changes	0.58 ± 0.27	-0.67 ± 0.41	**0.013**
Serum FBS (mg/dL)	Baseline	89.42 ± 3.24	93.40 ± 3.88	0.432
	Changes	6.64 ± 3.47	1.30 ± 4.21	0.330
Serum cholesterol (mg/dL)	Baseline	163.23 ± 6.84	174.26 ± 12.53	0.429
	Changes	-11.52 ± 10.06	-13.45 ± 10.35	0.894
Serum TG (mg/dL)	Baseline	162.38 ± 11.74	187.93 ± 19.75	0.259
	Changes	-29.81 ± 13.46	-21.69 ± 14.99	0.688
Serum HDL- C (mg/dL)	Baseline	32.42 ± 1.64	32.62 ± 1.41	0.929
	Changes	2.24 ± 2.18	9.11 ± 2.24	0.034
Serum LDL- C (mg/dL)	Baseline	101.96 ± 4.29	102.81 ± 4.976	0.897
	Changes	-5.54 ± 3.83	9.42 ± 5.30	0.025
Serum insulin (µm/mL)	Baseline	13.288 ± 1.53	11.01 ± 0.86	0.206
	Changes	0.66 ± 1.13	-0.29 ± 0.56	0.474
Serum hs-CRP (mg/L)	Baseline	2.96 ± 0.41	2.51 ± 0.35	0.419
	Changes	-1.10 ± 0.43	0.81 ± 0.49	**0.005**
Serum VLDL-C (mg/dL)	Baseline	32.47 ± 2.34	37.58 ± 3.94	0.259
	Changes	-5.96 ± 2.69	-4.33 ± 2.99	0.688

*The results are described as mean ± Standard Error (SE); # Independent sample *t*-test; FBS: Fasting Blood Sugar, hs-CRP: High Sensitive C Reactive Protein, HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein, VLDL: Very-Low-Density Lipoprotein, TG: Triglycerides

Table 4. Energy and macronutrients intakes at baseline and their changes during intervention*

		Omega 3group (N = 24)	Control group (N = 21)	*P*-value #
Total energy (kcal)	Baseline	1557.97 ± 128.47	1592.73 ± 139.17	0.855
	Changes	138.65 ± 144.88	-77.73 ± 170.37	0.336
Total protein (g)	Baseline	63.21 ± 6.58	63.91 ± 7.81	0.946
	Changes	-7.75 ± 8.29	-4.71 ± 10.11	0.816
Total fat (g)	Baseline	42.24 ± 6.18	36.98 ± 4.42	0.504
	Changes	3.53 ± 4.54	3.80 ± 6.19	0. 971
Total carbohydrate (g)	Baseline	238.32 ± 23.02	256.32 ± 25.05	0.599
	Changes	36.58 ± 25.35	-26.43 ± 27.37	0.098
Omega-6 fatty acids (g)	Baseline	13.18 ± 1.66	11.98 ± 1.66	0.614
	Changes	2.00 ± 2.22	4.02 ± 2.87	0.577
Omega-3 fatty acids (g)	Baseline	0.16 ± 0.05	0.10 ± 0.03	0.336
	Changes	0.001 ± 0.10	0.01 ± 0.06	0. 931

*The results are described as mean ± Standard Error (SE); # Independent sample t-test

Discussion

To the best of our knowledge, this study is the first clinical trial investigating the effects of Omega-3 fatty acids supplementation on serum irisin concentration in male CAD patients. This study showed that Omega-3 fatty acids supplementation could significantly increase serum irisin concentration and simultaneously diminish biomarkers of inflammation (a significant reduction in hs-CRP concentrations) at a dose of 720 mg EPA and 480 mg DHA, for 8 weeks. So, the main finding of this study would be that serum irisin increases as a result of Omega- 3 fatty acids consumption. However, compared with placebo, Omega- 3 fatty acids supplementation did not result in any significant changes in BMI, blood pressure, serum lipids with the exception of serum LDL, fasting blood glucose, fat mass, fat free mass, waist circumference, hip circumference, pulse rate (data not shown) or serum insulin.

There are some evidences suggesting that fish oil supplementation increases the serum LDL level,[20] but in the present study, serum LDL was reduced in the participants with Omega-3 fatty acids supplementation, in spite of statin therapy in 92% of participants. Some studies have demonstrated no changes in serum LDL associated with Omega-3 fatty acids consumption.[21] It seems that addition of Omega-3 fatty acids along with statin therapy could hasten serum LDL reduction.

The results of recent studies indicate that EPA and DHA play a crucial role in inflammation attenuation.[18] This study revealed that Omega−3 fatty acids supplementation had a significant effect on serum hs-CRP, as Omega-3 fatty acids supplementation reduces the serum hs-CRP levels. This finding is in accordance with the results of previous studies in CAD patient, demonstrating that there is an inverse association between Omega-3 fatty acids with hs-CRP concentration.[1] Also, Eftekhari, et al. reported a significant decrease in hs-CRP levels after supplementation with Omega-3 fatty acid for 2 months in atherosclerotic patients.[22] In a study by Vaughan, et al. Omega-3 fatty acids treatment of human rhabdomyosarcoma cell line for 24 and 48 hours resulted in induction of irisin and PGC-1α.[18] Induction of PGC-1α via increased expression of mitochondrial uncoupling proteins will result in enhancement of the metabolic rate and decrease in incidence of diabetes type 2 and obesity incidences.[18] Also, amplified expression of PGC-1α through glucose transporter 4 (GLUT4) and insulin sensitivity will result in a reduced incidence of diabetes.[18] Moreover, PGC-1α, through augmenting the expression of irisin, can result in increased metabolic rate.[18] Irisin is secreted into the circulation following proteolytic cleavage from its precursor, namely FNDC5.[23]

In a cross-sectional study on 1,115 community-living Chinese adults with central obesity and mean age of 53.2 (± 7.2) years, Yan, et al. found a negative association between serum irisin and insulin resistance indicators, such as fasting insulin, HbA1c, serum albumin/globulin ratio and abdominal adiposity (waist circumference) and no significant association between serum irisin and BMI, body fat OR muscle mass. They also reported increasing of serum irisin to be independently associated with reduced risks of metabolic syndrome and raised fasting plasma glucose.[24]

However, Liu, et al. suggest that circulating irisin concentrations are lower in diabetes mellitus type 2 compared with non-diabetic controls.[25] The study by Ming-Shien Wen, et al. indicated that chronic kidney disease patients in stage 5 had lower plasma irisin levels compared to sex-and age-matched healthy controls. Furthermore, another finding of this study was that irisin levels are independently associated with HDL cholesterol levels; there was a positive association between serum irisin and HDL cholesterol levels.[15] The results of a randomized controlled trial performed on 32 healthy, overweight coffee drinkers revealed no statistically significant change in serum irisin after eight weeks of coffee consumption. Also, the results of the mentioned study denoted that irisin levels are positively correlated with fat mass, waist circumference and CRP, a marker of inflammation.[26] A research project on obese Chinese adults with non-alcoholic fatty liver disease (NAFLD) suggested lower serum irisin in such patients. Likewise, this study showed an inverse association between serum irisin and triglyceride contents of the liver.[27] The strengths of the present study seem to be the novelty in terms of new metabolically important hormone as an outcome measure and its double-blind, randomized controlled design. The limitation of this study might be lack of coronary evaluation by angiography after the intervention assessing the impact of omega-3 supplementation on the extent of stenosis. As the mechanisms causing atherosclerosis are the same among patients with cardiovascular disease and the

impacts of Omega-3 fatty acids on these patients are similar, the results of the current study can be generalized to all men with cardiovascular disease.

In conclusion, it seems that Omega-3 fatty acids supplementation at a dose of 720 mg EPA and 480 mg DHA significantly increases serum irisin and also reduces serum LDL and inflammatory markers (hs-CRP) in CAD patients.

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