Ⓢ SAGE

Effect of Omega-3 Polyunsaturated Fatty Acids Supplementation on Body Composition and Circulating Levels of Follistatin-Like 1 in Males With Coronary Artery Disease: A Randomized Double-Blind Clinical Trial

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Abstract

Adipokines are mediators of body composition and are involved in obesity-related complications such as cardiovascular disease. Omega-3 supplementation has not been studied in the setting of body composition and follistatin-like 1 (FSTL1) levels in patients with coronary artery disease (CAD). This study aimed to investigate the effect of omega-3 polyunsaturated fatty acid (ω-3 PUFA) supplementation on body composition indices and serum levels of FSTL1 in CAD patients. A total of 42 male (aged 45–65 years) subjects with angiographically confirmed CAD were included in this randomized, double-blind, placebo-controlled trial study. The subjects were randomly divided into omega-3 and placebo groups. During the 8-week intervention, the omega-3 group received 1,200 mg of omega-3 daily, while the placebo group received paraffin. Before and after the study, anthropometric measurements and body composition components were taken; serum FSTL1 levels were assessed by an enzyme-linked immunosorbent assay (ELISA) kit. In the omega-3 group, a significant 27.6% increase in serum FSTL1 was seen after 8 weeks of intervention ($p = .001$), but no significant difference in posttreatment levels of FSTL1 was observed between the two groups ($p > .05$). At the end of the study, a significant decrease in low-density lipoprotein cholesterol (LDL-C; 94.29 ± 22.04 vs. 112.24 ± 24.5; $p = .01$) and high-sensitivity C-reactive protein (hs-CRP; 1.92 ± 0.79 vs. 3.19 ± 2.51; $p = .03$) concentration was detected between the two groups. Changes in fasting blood sugar, fasting insulin, body composition, and anthropometric parameters were not significant within and between the groups. Oral omega-3 might increase FSTL1 and decrease LDL-C and hs-CRP concentrations in CAD patients. However, omega-3 supplementation did not have any effect on FSTL1 levels between the groups.

Cardiovascular diseases (CVDs) are the main contributors to total global mortality and will continue to rise in the future (Maghbooli & Hossein-nezhad, 2015). Among the most prevalent causes of cardiovascular death is coronary artery disease (CAD; Yanicelli et al., 2015). CAD is caused by thrombotic occlusion of coronary arteries triggered by atherosclerotic plaque disruption, resulting in an activation of coagulation reactions (Uçar et al., 2011), and is one of the major causes of morbidity and mortality around the world (Abchee et al., 2006; Rai, Heidary-Moghadam, Asgari,

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Nowroozi, & Rasouli, 2014). Prevention and management of CVDs, including CAD, are of critical urgency.

Accumulating evidence indicates that the heart tissue secretes a variety of bioactive molecules, also known as cardiokines, that modulate the cellular processes in the heart in an autocrine, paracrine, or endocrine manner (Ogura et al., 2012; Walsh, 2009). One such protein created by the heart is follistatin-like 1 (FSTL1), a 308–amino acid extracellular glycoprotein (Wei et al., 2013). FSTL1, which is also named TSC-36, is a distant member of the follistatin family of proteins and was initially cloned from a mouse cell line (Chaly, Hostager, Smith, & Hirsch, 2014; Gorelik et al., 2012; Le Luduec et al., 2008; Widera et al., 2009). It functions as an extracellular antagonist of the transforming growth factor beta (TGF-β) superfamily cytokines (Oshima et al., 2009) and is expressed in different tissues such as skeletal muscle, adipose tissue, pituitary gland, testis, ovary, placenta, and brain (Fan et al., 2013; Hansen et al., 2013). The function of FSTL1 is not well defined; it has been reported that this cardiokine plays an important role in the regulation of cell survival, migration, differentiation, proliferation, metastasis, organ development, and carcinogenesis (Chaly et al., 2014). Moreover, recent studies indicate that FSTL1 has broad cardiovascular-protective activities (Ogura et al., 2012). FSTL1 expression is increased in the heart tissue in reaction to ischemia and hemodynamic stress (Widera et al., 2009) and is upregulated in heart failure, which promotes cardiac myocyte survival (Oshima et al., 2009). Accordingly, FSTL1 appears to be a clinically important secreted protein, which is involved in the pathophysiological responses to cardiovascular stress (Shimano et al., 2011), and could be considered as a clinical candidate for cardiac disease therapy agents (Walsh, 2009).

Cumulative evidence has revealed that routine consumption of cardioprotective nutrients in a healthy diet would significantly decrease the population risk of CAD (Cao et al., 2015). There is evidence supporting the advantageous effects of omega-3 polyunsaturated fatty acid (ω-3 PUFA) supplementation in patients with CVD (Khawaja, Gaziano, & Djoussé, 2014). The current study was designed to assess the effect of ω-3 PUFA supplementation on serum levels of FSTL1 and body composition components in patients with CAD.

Materials and Methods

Study Population

A total of 48 (aged 45–65 years) male subjects with angiographically confirmed CAD, with at least 50% stenosis in one or more epicardial coronary arteries, were eligible for the current randomized, double-blind, placebo-controlled trial study. All subjects were recruited from the cardiology clinic of Tehran Heart Center. The registered patients in the clinic were enrolled in this study according to the inclusion and exclusion criteria. Individuals were included if they met the following criteria: body mass index (BMI) ≥25 and no medical history of diabetes, renal disease, liver disease, thyroid dysfunction, and cancer. Prospective participants with any kind of myopathies, smokers (smoking was defined as smoking at least 5 cigarettes per day during the past 6 months), and those who were taking medicines such as warfarin, multivitamins, and omega-3 fatty acids or fish oil supplements were excluded. The participants were randomly divided into omega-3 and placebo groups by the permuted block randomization method. During the 8-week intervention, the omega-3 group received four softgels of ω-3 PUFA daily (2 softgels after lunch and 2 softgels after dinner), containing 480 mg docosahexaenoic acid (DHA) and 720 mg eicosapentaenoic acid (EPA), while the placebo group received four placebo softgels containing edible paraffin. The treatment and placebo softgels were identical in size and color. All patients were asked to maintain their routine physical activity and dietary habits and to report any change in the treatment protocol, use of medications, and dietary intake during the intervention. All participants provided written informed consent. Furthermore, the participants completed a self-administered questionnaire regarding demographic characteristics, health status, history of smoking, and participants' current medications. The study protocol was approved by the local ethical committee of Tehran University of Medical Sciences and was registered in ClinicalTrials.gov (NCT02382471).

Measurement of Body Composition and Anthropometric Parameters

Body composition of all subjects was assessed using a body composition analyzer (BC-418MA-Tanita, Middlesex, UK) by following the manufacturer's directions. The device calculates the body composition components, including body fat mass (FM), body fat percentage, visceral fat mass, truncal fat mass, fat-free mass (FFM), muscle mass, total body water (TBW), and BMI on the basis of data obtained by dual-energy X-ray absorptiometry using bioelectrical impedance analysis (BIA). In addition, at the baseline and after the intervention, patients' weight was assessed by a digital scale (Seca, Hamburg, Germany) in light clothing and barefoot with precision nearest to 0.1 kg. Moreover, height was measured by a seca stadiometer, with accuracy about 0.1 cm. BMI was also calculated as the weight (kg) divided by the square of the height (m). Waist circumference (WC) was measured in the smallest region of waist between the lower rib edge and the iliac ridge in the
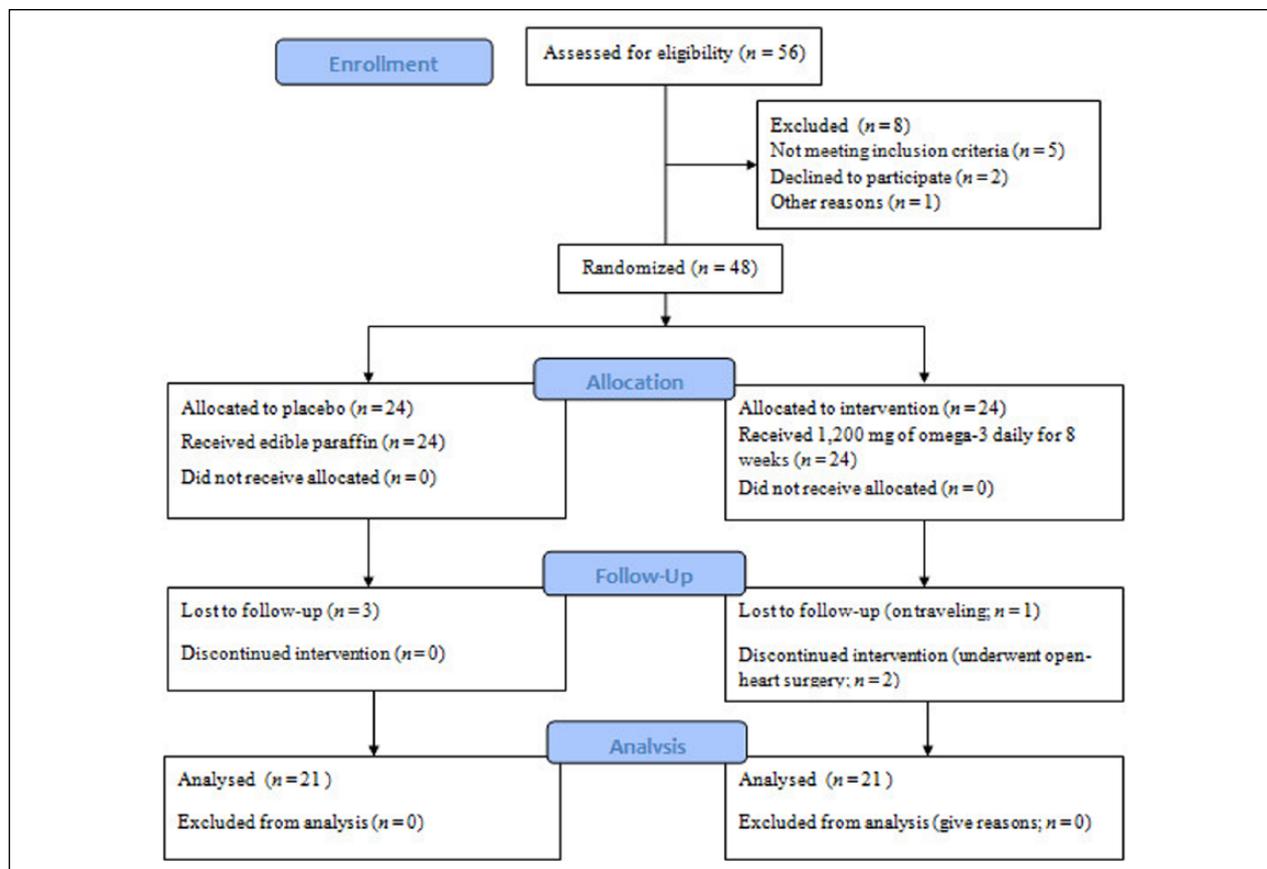
Figure 1. Flowchart of the study.

standing position by using a nonstretchable tape. Hip circumference (HC) was assessed in the widest part of the hip with accuracy nearest to 0.1 cm. Finally, the waist-to-hip ratio (WHR) was calculated by dividing WC by HC.

Measurement of the Biochemical Parameters

At the baseline and after the intervention, blood samples were obtained from all patients in the early morning after a 10- to 12-hr overnight fasting. All samples were centrifuged at 3,500 rpm for 10 min at -70°C, and separated stored sera at -20°C until analysis. Enzyme-linked immunosorbent assay (ELISA) kits were applied to assess the concentrations of high-sensitivity C-reactive protein (hs-CRP; Labor Diagnostika Nord, Nordhorn, Germany) and insulin (DiaMetra, Perugia, Italy) according to the manufacturers' instructions. Serum levels of triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C) were assessed with enzymatic methods by the use of commercial kits (Pars Azemun, Iran) and autoanalyzer system (Selectra E, Vitalab, Holliston, the Netherlands). Finally, serum follistatin-1 concentration was assessed by ELISA kit.

Statistical Analysis

The normality of data distribution was evaluated by the Kolmogorov-Smirnov test. In addition, paired t-test was applied for within-group comparisons (baseline vs. postintervention) and independent Student's t-test was conducted for comparisons between the two groups. Data are presented as mean ± standard deviation, and the level of significance was set at a probability of $\leq.05$ for all tests. Statistical analysis was performed using SPSS version 23.0 (SPSS, Chicago, IL, U.S.A).

Results

In total, 56 subjects were assessed for eligibility and 48 participants were randomized to intervention ($n = 24$) and placebo ($n = 24$) groups, and during the follow-up period, 6 participants dropped out. Details about the study are reported in the corresponding CONSORT 2010 flowchart (Figure 1). A total of 42 participants (intervention group: $n = 21$; placebo group: $n = 21$) were finally included in the present study. The mean age of intervention and placebo groups was 54.86 ± 6.05 and 57.76 ± 6.26 years, respectively. Tables 1 and 2 report baseline values and

Table 1. Patients' Baseline and End Point Characteristics in the Treatment and Control Groups.

	Omega-3 group (n = 21)		Placebo group (n = 21)		p*	P¶
	Before	After	Before	After		
Weight (kg)	81.83 ± 11.22	81.45 ± 11.25	77.36 ± 9.47	78.01 ± 9.23	.17	.28
BMI (kg/m²)	28.56 ± 3.45	28.42 ± 3.37	27.53 ± 3.43	27.74 ± 3.23	.33	.51
WC (cm)	99.54 ± 8.98	99.61 ± 8.71	97.81 ± 8.04	98.38 ± 8.06	.51	.63
HC (cm)	103.11 ± 6.20	102.47 ± 5.89	99.85 ± 5.01	100.19 ± 5.10	.06	.18
WHR	0.96 ± 0.05	0.97 ± 0.04	0.97 ± 0.05	0.97 ± 0.05	.47	.68
Fat mass (kg)	21.40 ± 7.34	20.96 ± 6.19	18.32 ± 6.30	18.56 ± 5.21	.15	.20
FFM (kg)	59.67 ± 6.51	60.35 ± 7.22	58.89 ± 6.38	58.95 ± 5.89	.69	.51
Trunk fat mass (kg)	12.87 ± 4.16	13.03 ± 3.98	11.33 ± 4.29	11.51 ± 3.55	.24	.22
FBS (mg/dL)	90.48 ± 16.09	96.45 ± 16.52	93.40 ± 17.79	94.71 ± 15.35	.57	.72
Insulin (μU/mL)	13.10 ± 7.79	13.94 ± 8.03	11.01 ± 3.98	10.72 ± 3.69	.28	.10
TC (mg/dL)	165.14 ± 33.6	148.95 ± 28.3	174.26 ± 57.4	160.81 ± 39.7	.53	.27
TG (mg/dL)	169.05 ± 58.4	134.26 ± 91.3	187.93 ± 90.5	166.24 ± 94.4	.42	.27
HDL (mg/dL)	32.76 ± 7.40	36.10 ± 7.46	32.62 ± 6.48	36.43 ± 8.35	.94	.89
LDL (mg/dL)	102.52 ± 21.4	94.29 ± 22.04	102.81 ± 22.8	112.24 ± 24.5	.96	.01
Hs-CRP (mg/L)	3.11 ± 2.12	1.92 ± 0.79	2.51 ± 1.62	3.19 ± 2.51	.30	.03

Note. BMI = body mass index; WC = waist circumference; HR = hip circumference; WHR = waist to hip ratio; FFM = fat free mass; TC = total cholesterol; TG = triglyseride; HDL = high density lipoprotein cholesterol; LDL = low density lipoprotein cholesterol; Hs-CRP = high sensitivity C reactive protein. Data are presented as mean ± SD.
p* = between groups p value at baseline; ¶ = between-groups p value after intervention.

Table 2. Changes from Baseline to End Point Measures of Follistatin-Like-1 Within Omega-3 and Placebo Groups and Between the Groups.

	Omega-3 group (n = 21)		Placebo group (n = 21)		Between groups at end	
Follistatin (μg/L)	Mean ± SD	p*	Mean ± SD	p*	Mean differences (95% CI)	p value
Baseline	45.75 ± 28.40		55.66 ± 72.12			
End	58.42 ± 34.67		49.74 ± 52.17		8.68 [−18.94, 36.31]	.56
Change: end − baseline	12.67 ± 15.63	.001	5.92 ± 20.68	.20		

Note. p* = within-group p value; data are presented as mean ± SD or mean (95% confidence interval).

changes after intervention in the two groups. At the baseline, there was no significant difference between the two groups (supplemented and control) in all investigated parameters (Tables 1 and 2). After the 8-week intervention, a significant decrease in LDL-C (94.29 ± 22.04 vs. 112.24 ± 24.5; p = .01) and hs-CRP (1.92 ± 0.79 vs. 3.19 ± 2.51; p = .03) concentration was identified between the two groups, while changes in TC, TG, and HDL were not significant within and between the groups. Furthermore, changes in fasting blood sugar, fasting insulin, body composition indices, and anthropometric characteristics were not significant within and between the investigated groups.

The mean serum FSTL1 was 45.75 ± 28.40 μg/L in omega-3 group and 55.66 ± 72.12 μg/L in the placebo group before supplementation. After 8 weeks intervention, the serum FSTL1 level increased significantly by 27.6% (p = .001) compared with baseline, while serum FSTL1 levels decreased by 10% in the placebo group (49.74 ± 52.17 vs. 55.66 ± 72.12; p = .20; Table 2). However, there was no significant difference in the variation of FSTL1 between the groups (p = 0.56; Table 2).

Discussion

Dietary supplementation therapy with ω-3 PUFA, including DHA, EPA, and α-linolenic acid has been reported as a promising approach for the primary and secondary prevention of CVD (Gilbert et al., 2015; Nestel et al., 2015). The therapeutic actions of ω-3 PUFA might be related to the lowering of serum TG (Patel et al., 2009), although the exact underlying mechanisms by which ω-3 PUFA affects CVD in humans have not yet been completely explained (Mostowik, Gajos, Zalewski, Nessler, & Undas, 2013). In

the present study, given the biological effects of ω-3 PUFA, the authors hypothesized that this nutrient might has some effects on body composition components and might increase the circulating level of FSTL1, a cardioprotective cardiokine (Görgens et al., 2013; Raschke & Eckel, 2013; Wei et al., 2015). Thus, this randomized, controlled trial was performed to determine the effect of ω-3 PUFA supplementation on body composition indices and serum levels of FSTL1 in patients with CAD.

As a main finding, this study identified a significant increase in serum FSTL1 concentrations in the treatment group following an 8-week therapy with 1,200 mg per day of ω-3 PUFAs. To the best of the authors' knowledge, the current study is the first to reveal increased FSTL1 levels in response to ω-3 PUFA supplementation in CAD patients. FSTL1 acts as an injury-induced secreted protein that protects against ischemic damage and has various positive functions in the heart and vasculature (Ouchi et al., 2010). The molecular mechanisms by which FSTL1 promotes cardiovascular cell protection and function are not completely understood. Data indicate that its cardiovascular protective effect is mediated by disconnected interacting protein 2 (DIP2A), which functions as an FSTL1 receptor on the cell surface of endothelial cells (Ouchi et al., 2010). Studies have demonstrated that FSTL1 can reduce myocardial ischemia reperfusion injury by inhibiting the inflammatory response and apoptosis through mechanisms in which adenosine monophosphate–activated protein kinase (AMPK) and bone morphogenetic protein-4 (BMP-4) play the central mediatory role (Liang et al., 2014; Ogura et al., 2012). Given the cardioprotective effects of FSTL1, and the present study findings indicating the positive effects of ω-3 PUFA on serum levels of FSTL1, the current study proposes that the beneficial effects of ω-3 PUFA on CVD prevention might be, at least in part, explained by the increased circulating levels of FSTL1.

This study identified no effect of administration of ω-3 PUFA on FFM, FM, trunk fat mass, and anthropometric parameters in patients with CAD. These findings are in agreement with previously conducted studies that have examined the effect of supplementation with long-chain PUFA on body composition indices in overweight and obese subjects (Crochemore, Souza, de Souza, & Rosado, 2012; DeFina, Marcoux, Devers, Cleaver, & Willis, 2010; Harden et al., 2014; Hill, Buckley, Murphy, & Howe, 2007; Krzymińska-Siemaszko et al., 2015;). The current study also examined the effects of ω-3 PUFA supplementation on lipid profile. The results revealed that ω-3 PUFA supplementation is associated with reduction in serum levels of LDL-C, but no significant change was observed in serum concentrations of TC, HDL-C, and TG. These findings are not in agreement with the majority of previous reports (Erkkilä

et al., 2014; Pirillo & Catapano, 2013; Weber & Raederstorff, 2000). Recent meta-analysis studies indicated that supplementation with PUFA and fish oil consumption decrease serum TG, improve HDL-C, and increase LDL-C levels (Balk et al., 2006; Bernstein, Ding, Willett, & Rimm, 2012). Harris (1997) analyzed 36 studies and reported that consumption of 3 to 4 g/day EPA + DHA could result in a plasma TG reduction by 24% in normolipemic patients and by 34% in hypertriacylglycerolemic subjects. Consistent with the current study, plasma HDL-C values were unaffected by ω-3 PUFA intake (Harris, 1997). In fact, ω-3 PUFA has a well-known effect on serum TG. ω-3 PUFA reduces plasma TG mainly via inhibition of TG and very–low-density lipoprotein apoB secretion from hepatic cells (Nestel et al., 1984). In addition, a recent mouse model study revealed that ω-3 PUFA supplementation as fish oil increases hepatic beta-oxidation of fatty acids and thus decreases hepatic fatty acid availability for TG synthesis and secretion (Wijendran & Hayes, 2004). These discrepancies might be due to the short follow-up period and relatively small sample size of the current study, which could limit the power to detect very moderate associations.

Chronic low-grade inflammation has a fundamental role in the initiation and development of CAD (Din, Newby, & Flapan, 2004), and hs-CRP independently predicts cardiovascular events (Pearson et al., 2003). Inhibition of inflammatory processes is generally cited as one of the primary mechanisms of ω-3 PUFA action in CVD (Madsen et al., 2007; Mori & Beilin, 2004;). Inconsistent with most studies (Dawczynski et al., 2013; Nigam et al., 2014), the present study revealed that ω-3 PUFA significantly reduces plasma levels of hs-CRP in patients with CAD. In the study by Madsen et al. (Madsen et al., 2007), supplementation with 5.2 g of ω-3 PUFA for 12 weeks had no significant effect on serum levels of hs-CRP in patients with a history of myocardial infarction. Therefore, the evidence regarding the effects of ω-3 PUFA on hs-CRP levels is inconclusive, indicating that additional studies are needed to elucidate the effect of ω-3 PUFA on hs-CRP levels in patients with CVD.

In summary, however, this study was limited by a relatively small sample size and a relatively short follow-up period, but for the first time, it was demonstrated that supplementation with 1,200 mg per day ω-3 PUFA for 8 weeks in patients with CAD improves FSTL1 levels, which might be a novel mechanism for the beneficial effects of ω-3 PUFA. Furthermore, the present study suggests that ω-3 PUFA supplementation might reduce serum levels of LDL-C and hs-CRP in CAD patients. Further studies should be performed at the cellular and molecular level to elucidate the effect of ω-3 PUFA on FSTL1 function.

Declaration of Conflicting Interests

Funding

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