Original Article

Metabolic Response to Omega-3 Fatty Acids and Vitamin E Co-Supplementation in Patients with Fibrocystic Breast Disease: A Randomized, Double-Blind, Placebo-Controlled Trial

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Abstract

Background: There is scarce data on the effects of omega-3 fatty acids and vitamin E co-supplementation on metabolic status in patients with fibrocystic breast disease (FBD). The current study was carried out to determine the effects of omega-3 fatty acids and vitamin E co-supplementation on metabolic status in patients with FBD.

Methods: A randomized clinical trial was conducted on 56 patients with FBD. Participants were randomly divided into two groups to receive either 1000 mg omega-3 fatty acids plus 400 mg vitamin E (n = 28) or placebo (n = 28) for 12 weeks. Fasting blood samples were taken at the beginning of the study and after 12 weeks of intervention to determine inflammatory factors, biomarkers of oxidative stress, and metabolic profiles.

Results: After 12 weeks of intervention, changes in serum high-sensitivity C-reactive protein (-2171.4 ± 3189.1 vs. +696.9 ± 2774.8 ng/mL, *P* = 0.001) and plasma nitric oxide (+1.8 ± 4.0 vs. -0.1 ± 2.4 μmol/L, *P* = 0.04) in supplemented women were significantly different from those in the placebo group. In addition, compared to the placebo group, subjects who consumed omega-3 fatty acids plus vitamin E supplements had significantly decreased serum insulin concentrations (-3.2 ± 6.5 vs. -0.2 ± 1.7 μIU/mL, *P* = 0.01), the homeostasis model of assessment-estimated insulin resistance (-0.8 ± 1.7 vs. -0.02 ± 0.4, *P* = 0.03), serum triglycerides levels (-11.5 ± 47.3 vs. +10.6 ± 24.3 mg/dL, *P* = 0.03) and VLDL-cholesterol (-2.3 ± 9.5 vs. +2.1 ± 4.9 mg/dL, *P* = 0.03), as well as increased quantitative insulin sensitivity check index (+0.01 ± 0.01 vs. +0.001 ± 0.007, *P* = 0.001) and HDL-cholesterol (+3.4 ± 6.0 vs. -1.3 ± 4.3 mg/dL, *P* = 0.001).

Conclusion: Overall, omega-3 fatty acids and vitamin E co-supplementation for 12 weeks had beneficial effects on inflammatory markers and metabolic profiles in patients with FBD.

Keywords: Fibrocystic breast disease, metabolic status, Omega-3 fatty acids, supplementation, vitamin E

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Introduction

Fibrocystic breast disease (FBD) is a noncancerous condition in which patients have painful lumps in their breasts.[1] The reported cumulative incidence rate over the reproductive life ranges between 45% and 70%.[2] Although the etiology of FBD has not been established, some evidence has implicated elevated estrogen levels, low progesterone levels, abnormal estrogen/progesterone ratio,[3] increased prolactin, growth factor, insulin, and thyroid hormone levels.[4] In addition, previous studies have reported that increased biomarkers of inflammation and oxidative stress play an important role in the pathogenesis of inflammatory, autoimmune and malignant diseases.[5–6]

Nowadays, there is growing interest to use omega-3 fatty acids or vitamin E in patients with FBD. A few studies have reported that omega-3 fatty acid concentration is lower in breast adipose tissue of women with breast cancer compared to those with benign conditions.[7–8] In another study, there was a significant difference in the total tocotrienol concentration between malignant and benign adipose tissue samples.[9] The beneficial effects of omega-3 fatty acids and vitamin E co-supplementation on metabolic profiles have been previously reported among women without FBD.[10] It is well established that combined omega-3 fatty acids and vitamin E supplementation can improve inflammation, oxidative stress, insulin resistance and lipid concentrations.[11–12] However, to our knowledge, there is scarce data on the effect of joint omega-3 fatty acid and vitamin E supplementation on biomarkers of inflammation and oxidative stress, glycemic status and lipid concentrations in patients with FBD.

Omega-3 fatty acids and vitamin E supplementation might affect inflammation, oxidative stress, glycemic control and lipid concentrations through their effects on increased activation of AMP-activated protein kinase,[13] decreased production of anti-inflammatory cytokines[14] and inhibited activation of nuclear factor-

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κB (NF-κB).[15–16] As there is evidence that omega-3 fatty acids and vitamin E co-supplementation may have anti-inflammatory, antioxidant and glucose-lowering effects, we hypothesized that omega-3 fatty acids and vitamin E co-supplementation might benefit patients with FBD. The aim of the current study was, therefore, to determine the effects of omega-3 fatty acids and vitamin E co-supplementation on biomarkers of inflammation and oxidative stress, and metabolic profiles in women with FBD.

Materials and Methods

Participants and sample size

The participants of this randomized double-blind placebo-controlled clinical trial were women with FBD aged 30–55 years who were referred to the Naghavi clinic affiliated to Kashan University of Medical Sciences (KUMS), Kashan, Iran between December 2015 and February 2016. Pregnant women and patients with metabolic disorders including thyroid dysfunction, diabetes or impaired glucose tolerance were excluded. On the basis of sample size formula suggested for randomized clinical trials, considering 5% type I error ($\alpha = 0.05$) and 20% type II error ($\beta = 0.20$; Power = 80%) and serum high-sensitivity C-reactive protein (hs-CRP) levels as key variable,[17] we used 2545.6 as SD and 2100.0 ng/mL as the change in mean (d) of serum hs-CRP levels as main variable. Based on this, we needed 24 subjects in each group. However, we recruited 28 subjects in each group (totally, 56 subjects) to compensate for the probable loss to follow-up.

Ethics statements

The present study protocol was confirmed in accordance with the principals of the Declaration of Helsinki and approved by the Research Council and the ethics committee of KUMS. Informed consent was taken from all participants. The current trial is registered at the Iranian registry of clinical trials (http://www.irct.ir: IRCT201510315623N55).

Study design and compliance

At first, participants were matched one-by-one based on body mass index (BMI) (<25 and ≥25 kg/m^2) and age (<25 and ≥25 years). Then, the matched participants were randomly assigned into two groups to receive combined omega-3 and vitamin E or placebo. The omega-3 fatty acids plus vitamin E supplements group (n = 28) received 1000 mg omega-3 fatty acids containing 400 mg α-Linolenic acid plus 400 IU vitamin E manufactured by Barij Essence Pharmaceutical Company (Kashan, Iran). The placebo (paraffin) group (n = 28) received one placebo capsule per day for 12 weeks which were identical in color, shape, size and package to the omega-3 fatty acids plus vitamin E capsules and produced by the same pharmaceutical company. Subjects were advised to keep their life style habits such as usual diet and levels of physical activity during the study period. Compliance with the intake of supplements or placebo capsules was determined by unused containers of the supplements and placebo capsules which were returned to the researchers. Furthermore, we sent a reminder to the patients' cell phones regarding consumption of supplements. Three-day dietary records (two week days and one weekend) at weeks 3, 6 and 9 of the treatment were obtained from each subject. To determine average daily macro- and micro-nutrient intakes of subjects, we used modified Nutritionist IV software (First Databank, San Bruno, CA). Physical activity was defined as metabolic equivalents (METs) in hours per day in this study. To quantify the METs for each subject, we multiplied the time (in hour per day) reported for each physical activity by its related METs coefficient in standard tables.[18]

Randomization

Randomization was achieved using computer-generated random numbers as blindness by a trained staff at the clinic.

Assessment of anthropometric measures

Weight and height (Seca, Hamburg, Germany) were assessed without shoes in light clothing in the gynecology clinic by a trained midwife, at the beginning of the study and after 3 months. BMI was calculated as weight (kg) divided by height squared (m^2).

Assessment of outcomes

Primary outcome was inflammatory markers. Secondary outcomes were biomarkers of oxidative stress, parameters of glucose homeostasis and lipid profiles.

Before the onset and after the end of the intervention, 10 mL blood samples were obtained from each patient at Naghavi Clinic laboratory in Kashan, Iran early in the morning after overnight fasting. Blood samples were immediately centrifuged (Hettich D-78532, Tuttlingen, Germany) at 3500 rpm for 10 min to separate serum. Then, the samples were stored at -70°C until analyzed at Naghavi Clinic laboratory. Serum hs-CRP concentrations were evaluated with commercial ELISA kit (LDN, Nordhorn, Germany) with inter- and intra-assay CVs of 3.4 to 6.1%, respectively. Plasma nitric oxide (NO) was measured using the Griess method,[19] total antioxidant capacity (TAC) using ferric reducing antioxidant power developed by Benzie and Strain,[20] total glutathione (GSH) according to Beutler *et al*.,[21] and malondialdehyde (MDA) concentrations using the thiobarbituric acid reactive substances spectrophotometric test.[22] All inter- and intra-assay coefficient variances (CVs) for NO, TAC, GSH and MDA concentrations were less than 5%. Available commercial kits were used to evaluate fasting plasma glucose (FPG), serum triglycerides, total-, VLDL-, LDL- and HDL-cholesterol concentrations (Pars Azmun, Tehran, Iran). All inter- and intra-assay CVs for FPG and lipid concentrations were less than 5%. Serum insulin levels were quantified using available ELISA kit (Monobind, California, USA) with inter- and intra-assay CVs of 2.9 to 5.1%, respectively. To determine the HOMA-IR and the quantitative insulin sensitivity check index (QUICKI), we used the suggested formulas.[23]

Statistical methods

We applied the Kolmogrov-Smirnov test to verify normal distribution of variables. The analyses were conducted based on intention-to-treat (ITT) approach. Missing values were treated based on Last-Observation-Carried-Forward method (LOCF)[24]; LOCF ignores whether the participant's condition was improving or deteriorating at the time of dropout but instead freezes outcomes at the value observed before dropout (i.e., last observation).[24] To detect differences in the subjects' general characteristics and dietary intakes between the two groups, independent sample *t*-test was applied. Independent sample *t*-test was used to determine the effects of omega-3 and vitamin E co-supplementation on biomarkers of inflammation and oxidative stress, glycemic control and lipid fractions. To adjust for confounders, ANCOVA

test was used to compare the mean changes of the outcome variables between the groups while adjusting for baseline values of biochemical variables, age and baseline BMI. In all analyses, *P*-value < 0.05 was considered statistically significant. The effect size (r^2) was calculated as the standardized mean difference between the two groups divided by SD of the population from which the groups were sampled. Statistical analyses were conducted using the Statistical Package for Social Science version 18 (SPSS Inc., Chicago, Illinois, USA).

Results

At baseline, we recruited 65 subjects; however, 9 subjects who did not meet his inclusion criteria were excluded from the study (Figure 1). Among subjects in the omega-3 fatty acid plus vitamin E as well as in the placebo groups, 3 subjects (withdrawn due to personal reasons) were excluded. Finally, 56 individuals [omega-3 fatty acids plus vitamin E (n = 28) and placebo (n = 28)] completed the trial. As analysis was carried out based on the ITT principle, all 56 individuals (28 in each group) were included in the final analysis. On average, the rate of compliance in our study was high, as more than 90% of capsules were taken throughout the study in both groups. No side effects were reported following the consumption of omega-3 fatty acids and vitamin E co-supplementation throughout the study.

No significant difference existed in mean age, height, weight and BMI before and after the treatment between the two groups (Table 1).

Comparison of total calorie intake, macro- and micro-nutrients between the two groups based on 3-day dietary records throughout the study showed no statistically significant difference (Data not shown).

After 12 weeks of intervention, changes in serum hs-CRP (-2171.4 ± 3189.1 vs. +696.9 ± 2774.8 ng/mL, *P* = 0.001) and plasma NO (+1.8 ± 4.0 vs. -0.1 ± 2.4 µmol/L, *P* = 0.04) in supplemented women were significantly different from those in the placebo group (Table 2). In addition, compared to the placebo group, subjects who consumed omega-3 fatty acids plus vitamin E supplements had significantly decreased serum insulin concentrations (-3.2 ± 6.5 vs. -0.2 ± 1.7 µIU/mL, *P* = 0.01), HOMA-IR (-0.8 ± 1.7 vs. -0.02 ± 0.4, *P* = 0.03), serum triglycerides levels (-11.5 ± 7.3 vs. +10.6 ± 24.3 mg/dL, *P* = 0.03) and VLDL-cholesterol (-2.3±9.5 vs. +2.1±4.9 mg/dL, P=0.03), as well as increased QUICKI (+0.01±0.01 vs. +0.001 ± 0.007, *P* = 0.001) and HDL-cholesterol (+3.4 ± 6.0 vs. -1.3 ± 4.3 mg/dL, *P* = 0.001). We did not find any significant change in other lipid concentrations or biomarkers of oxidative stress. Furthermore, on analysis without ITT approach, no significant change was observed in our findings.

When we adjusted the analysis for baseline values of biochemical parameters, age and baseline BMI, serum triglycerides (*P* = 0.07) and VLDL-cholesterol (*P* = 0.07) became non-significant, while FPG (*P* = 0.02) became statistically significant, and the other findings did not change (Table 3).

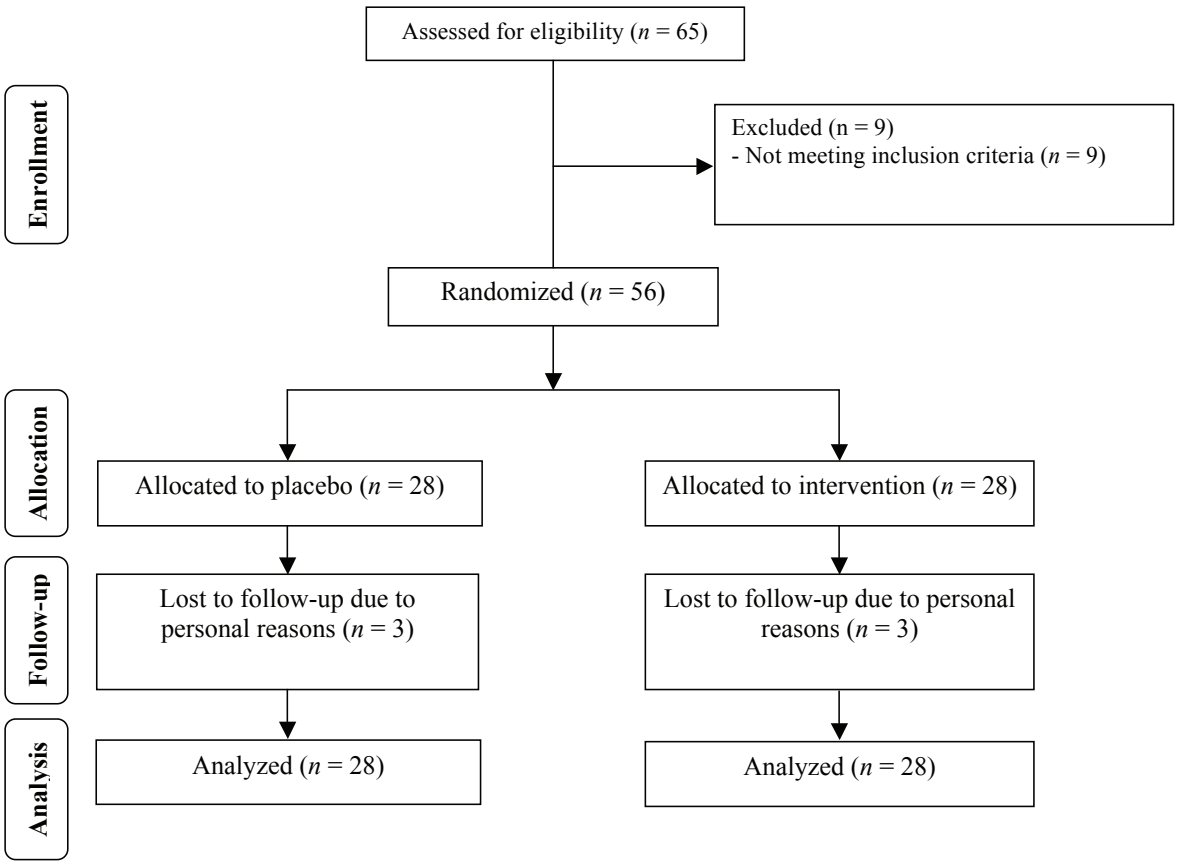

Figure 1. Summary of patient flow diagram.

Table 1. General characteristics of study participants[1]

	Placebo group (n = 28)	Omega-3 and vitamin E group (n = 28)	P[2]
Age (y)	47.6 ± 5.8	45.3 ± 7.2	0.18
Height (cm)	156.4 ± 5.6	156.3 ± 5.2	0.96
Weight at study baseline (kg)	72.0 ± 10.5	76.8 ± 12.7	0.13
Weight at end-of-trial (kg)	72.0 ± 10.2	77.1 ± 12.9	0.10
Weight change (kg)	0.01 ± 0.7	0.3 ± 1.4	0.35
BMI at study baseline (kg/m^2)	29.5 ± 4.3	31.4 ± 4.6	0.11
BMI at end-of-trial (kg/m^2)	29.5 ± 4.2	31.5 ± 4.7	0.10
BMI change (kg/m^2)	0.01 ± 0.3	0.1 ± 0.6	0.37

[1] Data are means± SDs. [2] Obtained from independent sample t-test.

Table 2. Biomarkers of inflammation and oxidative stress, and metabolic profiles at the study baseline and after 12-week intervention in women with fibrocystic breast disease who received either omega-3 fatty acids plus vitamin E supplements or placebo[1]

	Placebo group (n = 28)			Omega-3 and vitamin E group (n = 28)			P[2]
	Baseline	End-of-trial	Change	Baseline	End-of-trial	Change	
hs-CRP (ng/mL)	5344.9 ± 3933.8	6041.9 ± 4126.6	696.9 ± 2774.8	5506.4 ± 4416.8	3335.0 ± 2927.2	-2171.4 ± 3189.1	0.001
NO (µmol/L)	35.6 ± 2.3	35.5 ± 2.0	-0.1 ± 2.4	35.3 ± 2.5	37.1 ± 3.1	1.8 ± 4.0	0.04
TAC (mmol/L)	789.8 ± 176.87	734.5 ± 212.3	-55.4 ± 151.5	806.6 ± 111.2	799.7 ± 109.9	-6.9 ± 142.6	0.22
GSH (µmol/L)	524.8 ± 170.6	489.8 ± 193.9	-35.0 ± 201.1	497.9 ± 82.6	557.3 ± 161.3	59.5 ± 185.5	0.07
MDA (µmol/L)	1.9 ± 0.8	2.0 ± 0.7	0.1 ± 0.7	2.0 ± 0.5	1.8 ± 0.5	-0.2 ± 0.7	0.21
FPG (mg/dL)	84.9 ± 20.3	85.5 ± 17.8	0.6 ± 7.5	80.3 ± 15.6	76.6 ± 12.4	-3.7 ± 12.6	0.12
Insulin (µIU/mL)	12.2 ± 5.2	12.0 ± 5.1	-0.2 ± 1.7	14.8 ± 7.7	11.6 ± 4.6	-3.2 ± 6.5	0.01
HOMA-IR	2.5 ± 1.2	2.5 ± 1.3	-0.02 ± 0.4	3.1 ± 2.3	2.3 ± 1.1	-0.8 ± 1.7	0.03
QUICKI	0.33 ± 0.01	0.33 ± 0.02	0.001 ± 0.007	0.33 ± 0.02	0.34 ± 0.02	0.01 ± 0.01	0.001
Triglycerides (mg/dL)	127.8 ± 66.0	138.4 ± 66.9	10.6 ± 24.3	147.5 ± 73.4	136.0 ± 51.3	-11.5 ± 47.3	0.03
VLDL-cholesterol (mg/dL)	25.6 ± 13.2	27.7 ± 13.4	2.1 ± 4.9	29.5 ± 14.7	27.2 ± 10.3	-2.3 ± 9.5	0.03
Total cholesterol (mg/dL)	181.6 ± 37.5	176.9 ± 37.1	-4.7 ± 36.3	179.6 ± 34.2	185.4 ± 38.7	5.8 ± 25.6	0.21
LDL-cholesterol (mg/dL)	95.2 ± 32.8	89.7 ± 26.4	-5.5 ± 34.3	96.4 ± 26.4	101.0 ± 32.3	4.6 ± 25.1	0.21
HDL-cholesterol (mg/dL)	60.8 ± 8.5	59.5 ± 8.4	-1.3 ± 4.3	53.7 ± 7.6	57.2 ± 8.8	3.4 ± 6.0	0.001

1 All values are means± SDs. 2 Obtained from independent sample t-test.
FPG = fasting plasma glucose; GSH = total glutathione; HOMA-IR = homeostasis model of assessment-estimated insulin resistance; hs-CRP = high-sensitivity C-reactive protein; MDA = malondialdehyde; NO = nitric oxide; QUICKI = quantitative insulin sensitivity check index; TAC = total antioxidant capacity.

Table 3. Adjusted changes in metabolic variables in women with fibrocystic breast disease who received either omega-3 fatty acids plus vitamin E supplements or placebo.[1]

	Placebo group (*n* = 28)	Omega-3 and vitamin E group (*n* = 28)	95% CI	Effect size	P[2]
hs-CRP (ng/mL)	800.0 ± 486.7	-2274.5 ± 486.7	1664.47, 4484.61	0.27	<0.001
NO (µmol/L)	-0.04 ± 0.5	1.7 ± 0.5	-3.28, -0.29	0.10	0.02
TAC (mmol/L)	-48.8 ± 26.4	-13.4 ± 26.4	-111.91, 41.12	0.01	0.35
GSH (µmol/L)	-22.4 ± 34.0	46.8 ± 34.0	-167.91, 29.47	0.03	0.16
MDA (µmol/L)	0.1 ± 0.1	-0.2 ± 0.1	-0.07, 0.57	0.04	0.12
FPG (mg/dL)	1.3 ± 1.7	-4.5 ± 1.7	0.73, 10.84	0.09	0.02
Insulin (µIU/mL)	-0.6 ± 0.7	-2.9 ± 0.7	0.34, 4.23	0.09	0.02
HOMA-IR	-0.1 ± 0.2	-0.7 ± 0.2	0.06, 0.99	0.06	0.02
QUICKI	0.00 ± 0.002	0.01 ± 0.002	-0.01, -0.004	0.19	0.001
Triglycerides (mg/dL)	7.9 ± 6.3	-8.9 ± 6.3	-1.63, 35.7	0.06	0.07
VLDL-cholesterol (mg/dL)	1.58 ± 1.26	-1.77 ± 1.26	-0.32, 7.03	0.06	0.07
Total cholesterol (mg/dL)	-5.0 ± 5.7	6.1 ± 5.7	-27.61, 5.38	0.03	0.18
LDL-cholesterol (mg/dL)	-6.4 ± 5.1	5.4 ± 5.1	-26.38, 2.88	0.04	0.11
HDL-cholesterol (mg/dL)	-0.9 ± 1.1	3.2 ± 1.1	-7.32, -0.95	0.11	0.01

[1]All values are means± SDs.
[2]Obtained from analysis of covariance adjusted for baseline values+ age and baseline BMI.
FPG = fasting plasma glucose; GSH = total glutathione; HOMA-IR = homeostasis model of assessment-estimated insulin resistance; hs-CRP = high-sensitivity C-reactive protein; MDA = malondialdehyde; NO = nitric oxide; QUICKI = quantitative insulin sensitivity check index; TAC = total antioxidant capacity.

Discussion

Our findings demonstrated that omega-3 fatty acids and vitamin E co-supplementation for 12 weeks among women with FBD had beneficial effects on inflammatory factors, markers of insulin metabolism, triglycerides, and VLDL- and HDL-cholesterol levels; however, it did not affect the biomarkers of oxidative stress, FPG or other lipid profiles. To the best of our knowledge, this trial is the first evaluating the effects of omega-3 fatty acids and vitamin E co-supplementation on metabolic profiles in women with FBD. In the current study, the effect size of TAC, GSH, MDA, and total- and LDL-cholesterol was lower than 0.05, meaning that the score of the average person in the intervention group was lower than 0.05 standard deviations above the average person in the placebo group, and hence exceeds the scores of 50% of the placebo group. In addition, the effect size of NO, FPG, insulin, HOMA-IR, triglycerides and VLDL-cholesterol was nearly 0.1, indicating that the score of the average person in the intervention group was nearly 0.1 standard deviations above the average person in the placebo group, and hence exceeds the scores of 54% of the placebo group. The effect size of QUICKI was nearly 0.2, meaning that the score of the average person in the intervention group was nearly 0.2 standard deviations above the average person in the placebo group, and hence exceeds the scores of 58% of the placebo group. Finally, the effect size of hs-CRP was nearly 0.3, meaning that the score of the average person in the intervention group was nearly 0.3 standard deviations above the average person in the placebo group, and hence exceeds the scores of 62% of the placebo group. It must be kept in mind that in the current study, inflammatory markers constituted the primary outcome. Mean circulating levels of serum hs-CRP in study participants were above normal values (>3000 ng/mL). On the other hand, 42.9% and 46.4% of participants in the placebo and intervention groups had high insulin resistance (>2.5). Previous studies have demonstrated that increased inflammatory markers play an important role in the pathogenesis of autoimmune and malignant diseases.[5–6] Therefore, due to their beneficial effects on inflammatory markers and other metabolic profiles, omega-3 and vitamin E co-supplementation may be useful in decreasing FBD complications.

We found that taking combined omega-3 fatty acids and vitamin E supplements for 12 weeks in patients with FBD decreased serum hs-CRP and increased plasma NO levels, but it did not influence the biomarkers of oxidative stress. We have previously shown that joint omega-3 fatty acids and vitamin E supplementation for 12 weeks among hemodialysis (HD) subjects resulted in significant increases in plasma NO and TAC, and a significant decrease in plasma MDA levels compared with placebo; however, it did not influence serum hs-CRP and plasma GSH concentrations.[17] In addition, in another study by Desideri *et al*.[25], it was observed that 400 or 800 IU vitamin E administration among hypercholesterolemic patients for 8 weeks significantly increased NO levels. Likewise, supplementation with fish oil during pregnancy was associated with decreased maternal inflammatory cytokines.[26] Our previous study on patients with gestational diabetes mellitus (GDM) showed that supplementation with omega-3 fatty acids for 6 weeks resulted in reduced levels of plasma MDA, but unchanged plasma TAC and GSH concentrations.[27] In addition, taking 500 mg/day vitamin E for a 12-month period did not improve oxidative status of the HD patients.[28] The disparity in findings of the current study compared to others might reflect different study designs, difference in the study population, and the original omega-3 fatty acids from fish or flaxseed oil, dosage of used omega-3 fatty acids and vitamin E as well as duration of the trial. Increased inflammatory markers are involved in promotion of inflammatory responses and play an

important role in the pathogenesis of inflammatory and malignant diseases.[29] In addition, increased levels of inflammatory cytokines in cancer patients are associated with poor disease outcome.[30] Inflammatory markers are also key angiogenic molecules that may promote angiogenesis directly through stimulating endothelial cell proliferation as well as indirectly by modulating expression of other proangiogenetic factors.[31] Furthermore, changes in free radicals and lipid peroxidation have been reported in both blood and tissue of malignant breast tumor and benign breast disease.[32–33] Increased biomarkers of oxidative stress and lipid peroxidation are implicated in carcinogenic processes.[34] Therefore, due to their anti-inflammatory and anti-oxidative actions, omega-3 fatty acids and vitamin E may be useful in controlling FBD complications. The beneficial effects of omega-3 fatty acids and vitamin E supplementation on inflammatory factors may be due to their effects on decreased production of anti-inflammatory cytokines[14] and inhibited activation of NF-κB.[15–16] Furthermore, omega-3 fatty acids intake may increase the release of NO in smooth muscle cells as a consequence of decreased release of platelet-derived growth factor-like protein from platelets.[35]

Our study demonstrated that omega-3 fatty acids plus vitamin E administration for 12 weeks in patients with FBD resulted in significant reductions in serum insulin levels, HOMA-IR, serum triglycerides and VLDL-cholesterol concentrations, as well as significant increases in QUICKI and HDL-cholesterol levels compared with the placebo, while it did not affect FPG and other lipid fractions. Our previous study on GDM women indicated that omega-3 fatty acids and vitamin E co-supplementation for 6 weeks was associated with significant improvement in markers of insulin metabolism and a significant difference in triglycerides, and VLDL-, LDL- and HDL-cholesterol concentrations compared with the placebo, while it did not affect total cholesterol levels.[10] Likewise, Hutchins *et al*.[36] found that flaxseed oil consumption in overweight or obese individuals with pre-diabetes for 12 weeks resulted in a significant decrease in insulin concentrations and a significant rise in insulin sensitivity. In addition, another study by D'Adamo *et al*.[37] reported that high-dose (600 mg/day) vitamin E administration in obese children with non-alcoholic fatty liver disease for 6 months decreased HOMA-IR. A significant decrease in triglycerides concentrations without any change on other lipid profiles was also observed among non-alcoholic steatohepatitis patients following supplementation with omega-3 fatty acids for 3 months .[38] In contrast, some researchers did not observe such favorable effects of omega-3 fatty acids or vitamin E administration on glycemic control. For example, Barre *et al*.[39] have shown that the intake of flaxseed oil among subjects with T2DM for 12 weeks did not influence glycemic control. In another study conducted by Lemos *et al*.,[40] it was observed that supplementation with 2 g/day flaxseed oil among chronic hemodialysis patients for 120 days did not affect triglycerides levels. Positive relationships have been observed between markers of insulin resistance, including hyperglycemia[41] and hyperinsulinemia,[42] and breast cancer. Possible pathways have been proposed, mostly related to various hormonal factors, such as insulin-like growth factors, estrogen and their respective effect on cell growth, proliferation and differentiation.[43] Increased lipids and lipoproteins levels have been associated with breast cancer.[44] In addition, both benign and malignant proliferation of breast tissue in patients have been associated with changes in lipid and lipoprotein concentrations.[45] Dyslipidemia may reflect the effects of increased metabolic activity related to aggressive breast cancer, especially since breast cancer tends to be more aggressive in younger subjects.[45] Therefore, due to their favorable effects on insulin metabolism, triglycerides, VLDL- and HDL-cholesterol levels, combined omega-3 and vitamin E may be useful in decreasing metabolic complications related to FBD. Nonetheless, we believe that future studies are needed to confirm our findings. Omega-3 fatty acids and vitamin E intake may improve markers of insulin metabolism through elevated adiponectin levels[46] and protecting peripheral tissues and b-cells from free radical-mediated damage.[47] In addition, omega-3 fatty acids intake may affect lipid profiles through reduced jejunal secretion of apoB-48 by increasing its posttranslational degradation,[48] decreasing hepatic production of VLDL-cholesterol,[49] increased expression of hepatic peroxisome proliferator-activated receptor alpha (PPAR)-α and decreased expression of PPAR-γ.[50]

This research had some limitations. Due to budget constraints, we did not evaluate measurements of fatty acids profiles and vitamin E levels at the beginning and at the end of the trial. In addition, the sample size was small in the current study. Therefore, further studies with larger sample size are required to confirm our findings.

Overall, omega-3 fatty acids and vitamin E co-supplementation for 12 weeks among patients with FBD had beneficial effects on inflammatory markers and metabolic profiles.

Conflicts of interest: None declared.

Author contributions

ZA contributed to conception, design, statistical analysis and drafting of the manuscript. S-MM, SM, BS, JZ-R, MT, NM and BB contributed to conception, data collection and manuscript drafting. All authors read and approved the final version of the paper.

Clinical registration

www.irct.ir as IRCT201510315623N55.

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References

1. Rinaldi P, Ierardi C, Costantini M, Magno S, Giuliani M, Belli P, et al. Cystic breast lesions: sonographic findings and clinical management. J Ultrasound Med. 2010; 29(11): 1617 – 1626.
2. Plu-Bureau G, Le MG, Sitruk-Ware R, Thalabard JC. Cyclical mastalgia and breast cancer risk: results of a French cohort study. Cancer Epidemiol Biomarkers Prev. 2006; 15(6): 1229 – 1231.
3. Wang DY, Fentiman IS. Epidemiology and endocrinology of benign breast disease. Breast Cancer Res Treat. 1985; 6(1): 5 – 36.
4. Catalano S, Mauro L, Bonofiglio D, Pellegrino M, Qi H, Rizza P, et al. In vivo and in vitro evidence that PPARgamma ligands are antagonists of leptin signaling in breast cancer. Am J Pathol. 2011; 179(2): 1030 – 1040.
5. Sirotkovic-Skerlev M, Cacev T, Krizanac S, Kulic A, Pavelic K, Kapitanovic S. TNF alpha promoter polymorphisms analysis in benign and malignant breast lesions. Exp Mol Pathol. 2007; 83(1): 54 – 58.
6. Shrivastava A, Tiwari M, Sinha RA, Kumar A, Balapure AK, Bajpai

VK, et al. Molecular iodine induces caspase-independent apoptosis in human breast carcinoma cells involving the mitochondria-mediated pathway. J Biol Chem. 2006; 281(28): 19762 – 19771.
7. Maillard V, Bougnoux P, Ferrari P, Jourdan ML, Pinault M, Lavillonniere F, et al. N-3 and N-6 fatty acids in breast adipose tissue and relative risk of breast cancer in a case-control study in Tours, France. Int J Cancer. 2002; 98(1): 78 – 83.
8. Zhu ZR, Agren J, Mannisto S, Pietinen P, Eskelinen M, Syrjanen K, et al. Fatty acid composition of breast adipose tissue in breast cancer patients and in patients with benign breast disease. Nutr Cancer. 1995; 24(2): 151 – 160.
9. Nesaretnam K, Gomez PA, Selvaduray KR, Razak GA. Tocotrienol levels in adipose tissue of benign and malignant breast lumps in patients in Malaysia. Asia Pac J Clin Nutr. 2007; 16(3): 498 – 504.
10. Taghizadeh M, Jamilian M, Mazloomi M, Sanami M, Asemi Z. A randomized-controlled clinical trial investigating the effect of omega-3 fatty acids and vitamin E co-supplementation on markers of insulin metabolism and lipid profiles in gestational diabetes. J Clin Lipidol. 2016; 10(2): 386 – 393.
11. Asemi Z, Soleimani A, Bahmani F, Shakeri H, Mazroii N, Abedi F, et al. Effect of the omega-3 fatty acid plus vitamin E supplementation on subjective global assessment score, glucose metabolism, and lipid concentrations in chronic hemodialysis patients. Mol Nutr Food Res. 2016; 60(2): 390 – 398.
12. Rahmani E, Samimi M, Ebrahimi FA, Foroozanfard F, Ahmadi S, Rahimi M, et al. The effects of omega-3 fatty acids and vitamin E co-supplementation on gene expression of lipoprotein(a) and oxidized low-density lipoprotein, lipid profiles and biomarkers of oxidative stress in patients with polycystic ovary syndrome. Mol Cell Endocrinol. 2017; 439: 247 – 255.
13. Higuchi T, Shirai N, Saito M, Suzuki H, Kagawa Y. Levels of plasma insulin, leptin and adiponectin, and activities of key enzymes in carbohydrate metabolism in skeletal muscle and liver in fasted ICR mice fed dietary n-3 polyunsaturated fatty acids. J Nutr Biochem. 2008; 19(9): 577 – 586.
14. Hassan Eftekhari M, Aliasghari F, Babaei-Beigi MA, Hasanzadeh J. Effect of conjugated linoleic acid and omega-3 fatty acid supplementation on inflammatory and oxidative stress markers in atherosclerotic patients. ARYA Atheroscler. 2013; 9(6): 311 – 318.
15. Rossi A, Kapahi P, Natoli G, Takahashi T, Chen Y, Karin M, et al. Anti-inflammatory cyclopentenone prostaglandins are direct inhibitors of IkappaB kinase. Nature. 2000; 403(6765): 103 – 108.
16. Wu D, Han SN, Meydani M, Meydani SN. Effect of concomitant consumption of fish oil and vitamin E on production of inflammatory cytokines in healthy elderly humans. Ann N Y Acad Sci. 2004; 1031: 422 – 424.
17. Asemi Z, Soleimani A, Shakeri H, Mazroii N, Esmaillzadeh A. Effects of omega-3 fatty acid plus alpha-tocopherol supplementation on malnutrition-inflammation score, biomarkers of inflammation and oxidative stress in chronic hemodialysis patients. Int Urol Nephrol. 2016; 48(11): 1887 – 1895.
18. Ainsworth BE, Haskell WL, Whitt MC, Irwin ML, Swartz AM, Strath SJ, et al. Compendium of physical activities: an update of activity codes and MET intensities. Med Sci Sports Exerc. 2000; 32(9 Suppl): S498 – S504.
19. Tatsch E, Bochi GV, Pereira Rda S, Kober H, Agertt VA, de Campos MM, et al. A simple and inexpensive automated technique for measurement of serum nitrite/nitrate. Clin Biochem. 2011; 44(4): 348 – 350.
20. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem. 1996; 239(1): 70 – 76.
21. Beutler E, Gelbart T. Plasma glutathione in health and in patients with malignant disease. J Lab Clin Med. 1985; 105(5): 581 – 584.
22. Janero DR. Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. Free Radic Biol Med. 1990; 9(6): 515 – 540.
23. Pisprasert V, Ingram KH, Lopez-Davila MF, Munoz AJ, Garvey WT. Limitations in the use of indices using glucose and insulin levels to predict insulin sensitivity: impact of race and gender and superiority of the indices derived from oral glucose tolerance test in African Americans. Diabetes Care. 2013; 36(4): 845 – 853.
24. Lachin JM. Fallacies of last observation carried forward analyses. Clin Trials. 2016; 13(2): 161 – 168.
25. Desideri G, Marinucci MC, Tomassoni G, Masci PG, Santucci A, Ferri C. Vitamin E supplementation reduces plasma vascular cell adhesion molecule-1 and von Willebrand factor levels and increases nitric oxide concentrations in hypercholesterolemic patients. J Clin Endocrinol Metab. 2002; 87(6): 2940 – 2945.
26. Krauss-Etschmann S, Hartl D, Rzehak P, Heinrich J, Shadid R, Del Carmen Ramirez-Tortosa M, et al. Decreased cord blood IL-4, IL-13, and CCR4 and increased TGF-beta levels after fish oil supplementation of pregnant women. J Allergy Clin Immunol. 2008; 121(2): 464 – 470.
27. Jamilian M, Samimi M, Kolahdooz F, Khalaji F, Razavi M, Asemi Z. Omega-3 fatty acid supplementation affects pregnancy outcomes in gestational diabetes: a randomized, double-blind, placebo-controlled trial. J Matern Fetal Neonatal Med. 2016; 29(4): 669 – 675.
28. Antoniadi G, Eleftheriadis T, Liakopoulos V, Kakasi E, Kartsios C, Passadakis P, et al. Effect of one-year oral alpha-tocopherol administration on the antioxidant defense system in hemodialysis patients. Ther Apher Dial. 2008; 12(3): 237 – 242.
29. Bazzoni F, Beutler B. The tumor necrosis factor ligand and receptor families. N Engl J Med. 1996; 334(26): 1717 – 1725.
30. Nakashima J, Tachibana M, Ueno M, Miyajima A, Baba S, Murai M. Association between tumor necrosis factor in serum and cachexia in patients with prostate cancer. Clin Cancer Res. 1998; 4(7): 1743 – 1748.
31. Leek RD, Landers R, Fox SB, Ng F, Harris AL, Lewis CE. Association of tumour necrosis factor alpha and its receptors with thymidine phosphorylase expression in invasive breast carcinoma. Br J Cancer. 1998; 77(12): 2246 – 2251.
32. Gonenc A, Erten D, Aslan S, Akinci M, Simsek B, Torun M. Lipid peroxidation and antioxidant status in blood and tissue of malignant breast tumor and benign breast disease. Cell Biol Int. 2006; 30(4): 376 – 380.
33. Khanzode SS, Muddeshwar MG, Khanzode SD, Dakhale GN. Antioxidant enzymes and lipid peroxidation in different stages of breast cancer. Free Radic Res. 2004; 38(1): 81 – 85.
34. Gonenc A, Ozkan Y, Torun M, Simsek B. Plasma malondialdehyde (MDA) levels in breast and lung cancer patients. J Clin Pharm Ther. 2001; 26(2): 141 – 144.
35. Fox PL, DiCorleto PE. Fish oils inhibit endothelial cell production of platelet-derived growth factor-like protein. Science. 1988; 241(4864): 453 – 456.
36. Hutchins AM, Brown BD, Cunnane SC, Domitrovich SG, Adams ER, Bobowiec CE. Daily flaxseed consumption improves glycemic control in obese men and women with pre-diabetes: a randomized study. Nutr Res. 2013; 33(5): 367 – 375.
37. D'Adamo E, Marcovecchio ML, Giannini C, de Giorgis T, Chiavaroli V, Chiarelli F, et al. Improved oxidative stress and cardio-metabolic status in obese prepubertal children with liver steatosis treated with lifestyle combined with Vitamin E. Free Radic Res. 2013; 47(3): 146 – 153.
38. Nogueira MA, Oliveira CP, Ferreira Alves VA, Stefano JT, Rodrigues LS, Torrinhas RS, et al. Omega-3 polyunsaturated fatty acids in treating non-alcoholic steatohepatitis: A randomized, double-blind, placebo-controlled trial. Clin Nutr. 2016; 35(3): 578 – 586.
39. Barre DE, Mizier-Barre KA, Griscti O, Hafez K. High dose flaxseed oil supplementation may affect fasting blood serum glucose management in human type 2 diabetics. J Oleo Sci. 2008; 57(5): 269 – 273.
40. Lemos JR, Alencastro MG, Konrath AV, Cargnin M, Manfro RC. Flaxseed oil supplementation decreases C-reactive protein levels in chronic hemodialysis patients. Nutr Res. 2012; 32(12): 921 – 927.
41. Rapp K, Schroeder J, Klenk J, Ulmer H, Concin H, Diem G, et al. Fasting blood glucose and cancer risk in a cohort of more than 140,000 adults in Austria. Diabetologia. 2006; 49(5): 945 – 952.
42. Kabat GC, Kim M, Chlebowski RT, Khandekar J, Ko MG, McTiernan A, et al. A longitudinal study of the metabolic syndrome and risk of postmenopausal breast cancer. Cancer Epidemiol Biomarkers Prev. 2009; 18(7): 2046 – 2053.
43. Xue F, Michels KB. Diabetes, metabolic syndrome, and breast cancer: a review of the current evidence. Am J Clin Nutr. 2007; 86(3): s823 – s835.
44. Schreier LE, Berg GA, Basilio FM, Lopez GI, Etkin AE, Wikinski RL. Lipoprotein alterations, abdominal fat distribution and breast cancer. IUBMB Life. 1999; 47(4): 681 – 690.
45. Lane DM, Boatman KK, McConathy WJ. Serum lipids and apolipoproteins in women with breast masses. Breast Cancer Res Treat. 1995; 34(2): 161 – 169.
46. Wu JH, Cahill LE, Mozaffarian D. Effect of fish oil on circulating adiponectin: a systematic review and meta-analysis of randomized controlled trials. J Clin Endocrinol Metab. 2013; 98(6): 2451 – 2459.

47. Rafraf M, Bazyun B, Sarabchian MA, Safaeiyan A, Gargari BP. Vitamin E Improves Serum Paraoxonase-1 Activity and Some Metabolic Factors in Patients with Type 2 Diabetes: No Effects on Nitrite/Nitrate Levels. J Am Coll Nutr. 2016; 35(6): 521 – 528.
48. Levy E, Spahis S, Ziv E, Marette A, Elchebly M, Lambert M, et al. Overproduction of intestinal lipoprotein containing apolipoprotein B-48 in Psammomys obesus: impact of dietary n-3 fatty acids. Diabetologia. 2006; 49(8): 1937 – 1945.
49. Miyoshi T, Noda Y, Ohno Y, Sugiyama H, Oe H, Nakamura K, et al. Omega-3 fatty acids improve postprandial lipemia and associated endothelial dysfunction in healthy individuals - a randomized cross-over trial. Biomed Pharmacother. 2014; 68(8): 1071 – 1077.
50. Kim do Y, Kim J, Ham HJ, Choue R. Effects of d-alpha-tocopherol supplements on lipid metabolism in a high-fat diet-fed animal model. Nutr Res Pract. 2013; 7(6): 481 – 487.