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The Effects of Omega-3 and Vitamin E Co-supplementation on Carotid Intima-media Thickness and Inflammatory Factors in Patients with Polycystic Ovary Syndrome

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ABSTRACT

Objectives: We sought to evaluate the effects of omega-3 and vitamin E co-supplementation on carotid intima-media thickness (CIMT) and inflammatory factors in patients with polycystic ovary syndrome (PCOS). **Methods:** This randomized, double-blind, placebo-controlled trial was done among 60 women with PCOS. Participants were randomly assigned into two groups (n = 30 each group) and assigned to take either 1000 mg omega-3 plus 400 IU vitamin E supplements or a placebo for 12 weeks. **Results:** Compared with placebo, omega-3 and vitamin E co-supplementation led to significant decreases in maximum levels of left CIMT (-0.006 ± 0.006 vs. $+0.002 \pm 0.007$ mm, $p < 0.001$), mean levels of left CIMT (-0.005 ± 0.006 vs. $+0.002 \pm 0.010$ mm, $p = 0.010$), maximum levels of right CIMT (-0.006 ± 0.010 vs. $+0.006 \pm 0.010$ mm, $p = 0.010$), and mean levels of right CIMT (-0.005 ± 0.005 vs. $+0.001 \pm 0.010$ mm, $p = 0.020$). Change in high-sensitivity C-reactive protein (hs-CRP) (-390.6 ± 942.9 vs. $+237.0 \pm 754.3$ ng/mL, $p = 0.006$) was significantly different between the supplemented patients and placebo group. We did not observe any significant effect in plasma nitric oxide (NO) values following supplementation with omega-3 plus vitamin E compared with the placebo. **Conclusions:** Co-supplementation with omega-3 and vitamin E for 12 weeks among patients with PCOS had beneficial effects on CIMT and serum hs-CRP values, but unchanged NO values.

Polycystic ovary syndrome (PCOS) is a reproductive and metabolic disorder correlated with several risk factors for atherosclerosis.¹ Previous studies have shown that the prevalence of cardiovascular disease (CVD) in subjects with PCOS is high compared with the normal population.^{2,3} Evaluation of preclinical vascular disease using noninvasive tests in patients with PCOS with greater carotid intima-media thickness (CIMT) has demonstrated that there is an increased tendency to atherosclerosis in these patients compared with healthy controls.^{4,5} Also, some studies have indicated that increased CIMT as a noninvasive marker is a powerful predictor of coronary events and is associated with traditional cardiovascular risk factors, including age,

obesity, and metabolic profiles especially increased inflammatory markers.^{6,7}

Antiatherogenic effects such as improving endothelial function, inhibiting platelet aggregation, and reducing levels of triglycerides of omega-3 were previously reported.⁸ A cross-sectional study observed that higher consumption of omega-3 was associated with a lower prevalence odds of carotid plaques and a lesser thickness of segment-specific CIMT.⁹ However, no significant effect on CIMT was seen following supplementation with 1 g of omega-3 over 4.9 years in people with CVD and/or CVD risk factors and dysglycemia.¹⁰ Several large observational and arterial imaging studies have demonstrated that daily consumption of 100 IU of vitamin E for at least two years decreased atherosclerosis progression and

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CVD event rates.^{11,12} However, in another study, vitamin E supplementation (400 IU) in healthy men and women at low risk for CVD did not reduce the progression of CIMT over a three-year period.¹³

The effects of omega-3 and vitamin E co-supplementation on metabolic profiles have been investigated previously.^{14,15} Omega-3 and vitamin E co-supplementation seems to work better than single supplementation. The effects of omega-3 and/or vitamin E supplements on human atherosclerosis progression were evaluated in a few small studies in subjects without PCOS, which were inconclusive. The current investigation was, therefore, done to assess the impacts of omega-3 and vitamin E co-supplementation on CIMT and inflammatory factors in patients with PCOS.

METHODS

This randomized, double-blind, placebo-controlled clinical trial, registered on the Iranian website for registration of clinical trials (<http://www.irct.ir:IRCT201511015623N57>) was conducted among 60 subjects with PCOS diagnosed according to the Rotterdam criteria,¹⁶ aged 18–40 years old who were referred to the Naghavi Clinic in Kashan, Iran, between June 2016 and October 2016. We excluded pregnant women and those with endocrine diseases and no hormonal treatments in the six months before the study. This trial was approved by the ethics committee of Kashan University of Medical Sciences (KAUMS), and informed consent was taken from all subjects. Subjects were randomly divided into two groups ($n = 30$ each group) to receive either 1000 mg omega-3 from flaxseed oil containing 400 mg α -linolenic acid plus 400 IU vitamin E supplements or a placebo for 12 weeks. The supplement and placebo (paraffin) were provided by Barij Essence Pharmaceutical Company (Kashan, Iran).

To evaluate compliance, we counted the remaining supplements. To increase compliance, all women received short messages every day reminding them to take the capsules.

We considered CIMT as primary outcome measurement and inflammatory parameters as secondary outcomes measurements.

CIMT measurements (maximum and mean of left and right CIMT) were done in patients at the 2 cm distance of the common carotid bifurcation

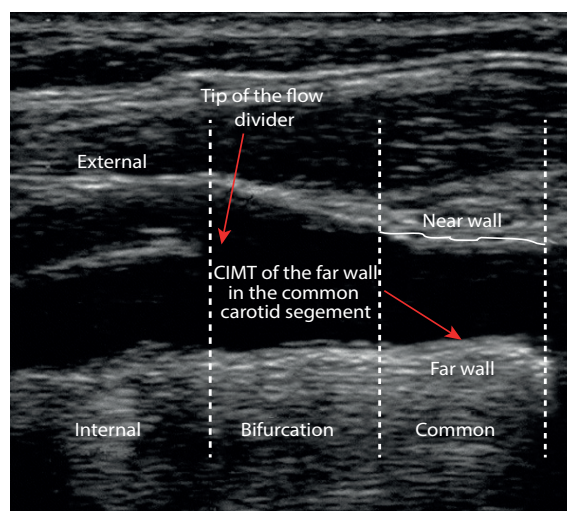


Figure 1: Placement of the ultrasonographic calipers to measure carotid intima-media thickness (CIMT).

[Figure 1], by the same sonographer, at baseline and after the 12-week intervention using a Doppler ultrasonography device (Samsung Medison V20, Korea) with linear multi-frequencies of 7.5- to 10-MHz probe. Reproducibility information was obtained from the duplicate ultrasound examinations at baseline and the end of the trial. The mean \pm standard deviation (SD) difference in common CIMT between the two baseline measurements (screening visit and randomization visit) was 0.0004 ± 0.058 mm. The mean absolute mean difference was 0.037 ± 0.042 mm. The intra- and interobserver coefficient variances (CVs) for the repeated measurements of mean CIMT were 6.5% and 9.6%, respectively. Furthermore, the intra- and interobserver CVs for the repeated measurements of maximum CIMT were 7.0% and 10.5%, respectively. All CIMT (mean and maximum thickness) measurements were evaluated blindly by a single experienced ultrasonographer.

Fasting blood samples (10 mm) were collected at baseline and after the 12-week treatment. Serum high-sensitivity C-reactive protein (hs-CRP) values were quantified by an ELISA kit (LDN, Nordhorn, Germany) with inter- and intra-assay CVs of lower than 7.0%. Plasma nitric oxide (NO) values were evaluated using the Griess method¹⁷ with inter- and intra-assay CVs $< 5.0\%$.

To establish normal data distribution, we used the Kolmogorov-Smirnov test. To establish differences in anthropometric measures between the two groups, we applied the independent samples

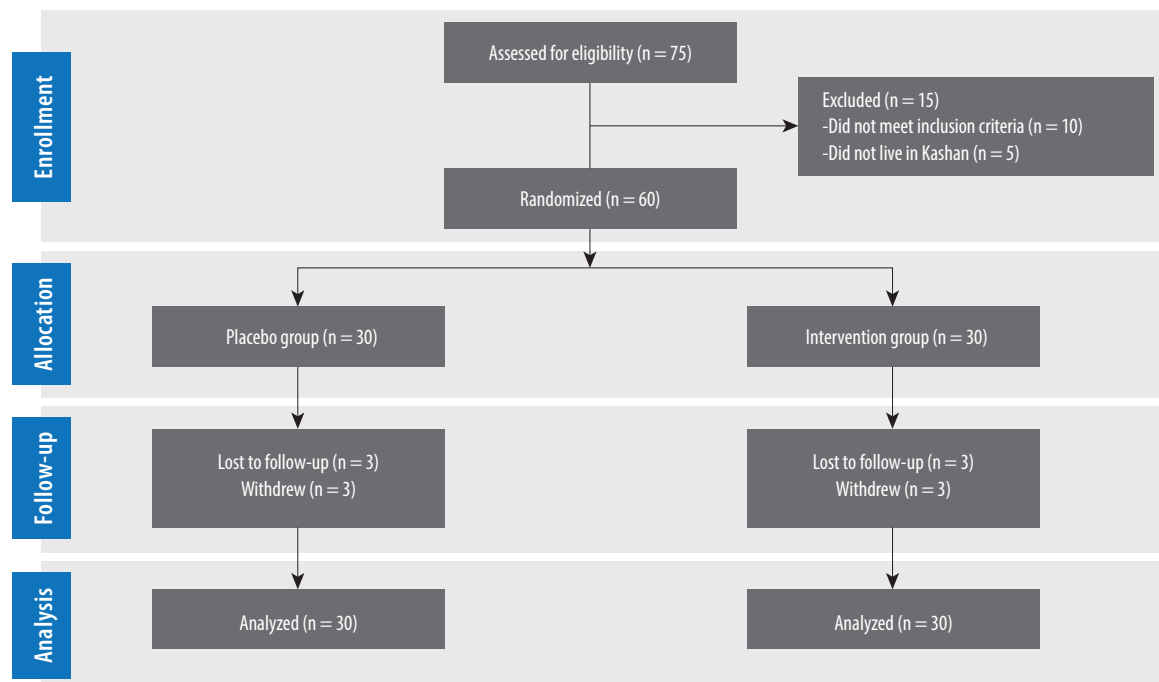


Figure 2: Patient flow diagram.

t-test. The intention-to-treat (ITT) analysis of the primary study end-point was applied to all randomly allocated subjects. To determine the effects of omega-3 and vitamin E co-supplements on CIMT and inflammatory markers, we used one-way repeated measures ANOVA. To evaluate for several confounders, we adjusted all analyses for baseline values, age, and baseline body mass index (BMI) to avoid potential bias using ANCOVA. A *p*-value < 0.050 was considered statistically significant. All data entry and statistical analyses were conducted using the SPSS Statistics (SPSS Inc. Released 2009. PASW Statistics for Windows, Version 18.0. Chicago: SPSS Inc.).

We used a randomized clinical trial sample size calculation formula where type one (α) and type two (β) errors were 0.05, and 0.20 (power = 80%), respectively. According to a previous trial,¹⁸ we used 0.18 mm as SD and 0.15 mm as the change in mean (effect size) of CIMT as a main variable. Based on the sample size calculation formula, we needed 25 subjects in each group. After considering five dropouts in each group, the final sample size was 30 subjects in each group.

RESULTS

Three patients in the omega-3 and vitamin E co-supplements group and three in the placebo

group withdrew from the study due to personal reasons and, therefore, did not complete the trial [Figure 2]. However, all 60 subjects were included in the final analysis using the ITT principle.

There were no significant differences between the two groups in mean age, height, weight, and BMI at baseline, and changes in weight and BMI at the end of the study (data not shown).

Omega-3 and vitamin E co-supplementation led to significant decreases in maximum levels of left CIMT (-0.006 ± 0.006 vs. $+0.002 \pm 0.007$ mm, $p < 0.001$), mean left CIMT levels (-0.005 ± 0.006 vs. $+0.002 \pm 0.010$ mm, $p = 0.010$), maximum levels of right CIMT (-0.006 ± 0.010 vs. $+0.006 \pm 0.010$ mm, $p = 0.010$), and mean right CIMT levels (-0.005 ± 0.005 vs. $+0.001 \pm 0.010$ mm, $p = 0.020$) after the 12-week intervention compared with placebo [Table 1]. Change in hs-CRP (-390.6 ± 942.9 vs. $+237.0 \pm 754.3$ ng/mL, $p = 0.006$) was significantly different between the intervention and placebo group. We did not observe any significant effect on NO values between the two groups at the end of the study.

There was a significant difference in baseline levels of NO ($p = 0.080$) between the two groups. When we adjusted the analysis for baseline values of biochemical parameters, age and baseline BMI, mean right CIMT ($p = 0.070$) became non-significant, while other findings did not alter [Table 2].

Table 1: Carotid intima-media thickness (CIMT) and inflammatory markers at baseline and 12 weeks after the intervention in patients with polycystic ovary syndrome.

	Placebo group, (n = 30)			Intervention group, (n = 30)					
	Baseline	At 12-weeks	Change	p-value	Baseline	At 12-weeks	Change	p-value ¹	p-value ²
Mean left CIMT, mm	0.47 ± 0.04	0.47 ± 0.04	0.002 ± 0.010	0.400	0.46 ± 0.02	0.45 ± 0.03	-0.005 ± 0.006	< 0.001	0.010
Maximum left CIMT, mm	0.56 ± 0.05	0.57 ± 0.05	0.002 ± 0.007	0.050	0.55 ± 0.03	0.55 ± 0.03	-0.006 ± 0.006	< 0.001	< 0.001
Mean right CIMT, mm	0.46 ± 0.03	0.46 ± 0.03	0.001 ± 0.010	0.600	0.48 ± 0.05	0.47 ± 0.05	-0.005 ± 0.005	< 0.001	0.020
Maximum right CIMT, mm	0.56 ± 0.04	0.56 ± 0.03	0.006 ± 0.010	0.040	0.57 ± 0.05	0.57 ± 0.05	-0.006 ± 0.010	0.004	0.010
hs-CRP, ng/mL	2646.7 ± 1492.3	2883.7 ± 1488.9	237.0 ± 754.3	0.090	2877.9 ± 2095.5	2487.3 ± 1673.1	-390.6 ± 942.9	0.030	0.006
NO, µmol/L	46.0 ± 6.0	46.1 ± 5.9	0.1 ± 2.6	0.810	49.6 ± 2.3	51.3 ± 4.7	1.7 ± 4.7	0.060	0.110

Data presented as mean ± standard deviation.

¹p-values represent paired-samples t-test.

²p-values represent the time × group interaction (computed by analysis of the one-way repeated measures ANOVA).

hs-CRP: high-sensitivity C-reactive protein; NO: nitric oxide.

DISCUSSION

To the best of our knowledge, this study is the first of its kind. We evaluated the beneficial effects of omega-3 and vitamin E co-supplementation on CIMT and inflammatory parameters among patients with PCOS. We found that co-supplementation with omega-3 and vitamin E for 12 weeks in patients with PCOS had beneficial effects on CIMT and hs-CRP levels, but did not affect NO values.

Omega-3 and vitamin E co-supplementation resulted in a significant reduction in maximum and mean levels of left and right CIMT in patients with PCOS compared with placebo. Another study observed an inverse relationship between fetal growth and arterial wall thickness in childhood, which can be prevented by dietary omega-3 supplementation in the first five years of life.¹⁹ In addition, supplementation with 3 g/day omega-3 for six months in patients undergoing hemodialysis decreased CIMT.¹⁸ The favorable effectiveness of omega-3 on CIMT progression in people with combined hyperlipoproteinemia was also reported.²⁰ Co-supplementation with vitamins E (136 IU/day) and C (250 mg/day) in hypercholesterolemic men for six years reduced common carotid artery (CCA)-IMT.²¹ Our findings are inconsistent with the results of previous studies examining the effects on carotid and coronary atherosclerosis,²²⁻²⁴ as well as a recent meta-analysis of the effects of omega-3 fatty acids on clinical outcomes.²⁵ In addition, vitamin E supplementation (1200 IU/day) for two years in patients with coronary artery disease reduced parameters of inflammation and oxidative stress, but had no significant effect on CIMT.²⁶ It must be kept in mind that beneficial effects of omega-3 fatty acids or vitamin E on CIMT in most previous studies were observed after six months, while in our study, the beneficial effects of omega-3 and vitamin E co-supplementation on CIMT were seen after three months. This may be due to co-supplementation working better than single supplementation. However, data on CVD events in women with PCOS are limited, but a recent meta-analysis demonstrated that women with PCOS had twice the relative risk of CVD or stroke than control patients.²⁷ In a meta-analysis study, CIMT artery in women with PCOS was significantly higher than healthy women. CIMT has been widely used as a surrogate index of atherosclerosis and CVD events.^{28,29} Omega-3 may mitigate the adverse effects of reduced Δ-5

Table 2: Adjusted changes in carotid intima-media thickness (CIMT) and inflammatory markers in patients with polycystic ovary syndrome.

Variables	Placebo group (n = 30)	Intervention group (n = 30)	p-value ¹
Mean left CIMT, mm	0.002 ± 0.002	-0.005 ± 0.002	0.020
Maximum left CIMT, mm	0.003 ± 0.001	-0.006 ± 0.001	< 0.001
Mean right CIMT, mm	0.001 ± 0.002	-0.005 ± 0.002	0.070
Maximum right CIMT, mm	0.005 ± 0.003	-0.005 ± 0.003	0.007
hs-CRP, ng/mL	206.6 ± 140.1	-360.2 ± 140.1	0.006
NO, µmol/L	-0.05 ± 0.7	1.8 ± 0.7	0.080

Data presented as means ± standard deviation. Values are adjusted for baseline values, age, and body mass index at baseline.

¹Obtained using ANCOVA.

hs-CRP: high-sensitivity C-reactive protein; NO: nitric oxide.

desaturase activity,¹⁹ which in turn may improve CIMT. Moreover, omega-3 supplementation appears to have a cardioprotective effect in adults, potentially through effects on lipid fractions, endothelial function, and/or antiarrhythmic and anti-inflammatory actions.³⁰ Vitamin E intake due to its antioxidant and anti-inflammatory effects may improve CIMT.²¹

Omega-3 and vitamin E co-supplementation in patients with PCOS for 12 weeks was associated with a significant reduction in hs-CRP levels, but unchanged NO values. Supporting our results, Rizza et al,³¹ observed that supplementation with omega-3 (2 g/day) significantly improved endothelial function and decreased pro-inflammatory markers in offspring of patients with type 2 diabetes mellitus for 12 weeks. In addition, another meta-analysis study observed that marine-derived omega-3 supplementation had a significant CRP lowering effect.³² We have previously shown beneficial effects of six-weeks omega-3 supplementation (1000 mg/day) on hs-CRP levels among women with gestational diabetes mellitus (GDM).³³ Likewise, three weeks of supplementation with 200 mg/day vitamin E caused a significant decrease in CRP among patients with coronary heart disease.³⁴ The results of a meta-analysis study have shown that supplementation with vitamin E resulted in decreased levels of CRP.³⁵ However, omega-3 administration for six weeks in subjects with visceral obesity did not cause any significant change in hs-CRP values.³⁶ Co-supplementation with omega-3 and vitamin E for six weeks among patients with GDM showed no significant changes in hs-CRP levels.³⁷ Previous studies reported a 4.4-fold increase in the relative risk for CVD comparing the highest and lowest quartiles of

CRP, while this increase is only 2.4-fold comparing the quartiles of cholesterol.^{38,39} CRP stimulates mononuclear cells to release tissue factors which are important in the initiation of coagulation reactions, complement activation, and neutralization of platelet-activating factor, which in turn promote thrombotic response.³⁹ The beneficial effects of omega-3 and vitamin E co-supplementation on inflammatory cytokines may be due to their effects on decreased production of anti-inflammatory parameters⁴⁰ and inhibited activation of nuclear factor kappa B.^{41,42}

Our study had some limitations. Due to funding limitations, we did not assess the effects of omega-3 and vitamin E co-supplementation on the measurements of fatty acids profiles and vitamin E. In addition, this study was a relatively short intervention. Long-term interventions might result in better changes in mean levels of CIMT.

CONCLUSION

Co-supplementation with omega-3 and vitamin E for 12 weeks in patients with PCOS had beneficial effects on CIMT and serum hs-CRP concentration, but unchanged NO values.

Disclosure

The authors declared no conflicts of interest. The study was founded by a grant from the Vice-chancellor for Research, KAUMS, Iran.

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