



Low-Dose Omega-3 Fatty Acid and Vitamin D for Anthropometric, Biochemical Blood Indices and Respiratory Function. Does it work?

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Abstract: Omega-3 fatty acids and vitamin D3 have beneficial effects on different blood, cardiovascular parameters and physical performance. However, the effect of low-dose omega-3 fatty acid supplementation remains unclear. 84 office workers aged 40–60 years, participated in a 16-week open, randomized, placebo-controlled, parallel-group study. The experimental group received 330 mg of omega-3 fatty acid and 0.005 mg (200 IU) of vitamin D3 per day and the control group received placebo. Anthropometric, biochemical blood and respiratory indices were measured at 12 and 16 weeks. Body mass (BM) and body mass index (BMI) significantly reduced in both the experimental (BM from 74.4 ± 13.04 to 73.2 ± 13.02 kg, $p < 0.001$; BMI from 25.8 ± 4.1 to 25.4 ± 4.3 kg/m², $p < 0.001$) and the placebo groups (BM from 69.5 ± 11.1 to 68.7 ± 11.4 kg, $p < 0.05$; BMI from 24.1 ± 4.0 to 23.8 ± 4.2 kg/m², $p < 0.05$). Omega-3 fatty acid supplementation significantly improved glucose (from 5.12 ± 0.55 to 4.97 ± 0.62 mmol/l; $p = 0.05$), total cholesterol (from 5.86 ± 1.0 to 5.32 ± 1.55 mmol/l; $p = 0.003$), and vitamin D levels (from 35.07 ± 21.65 to 68.63 ± 25.94 nmol/l; $p = 0.000$). Maximal oxygen consumption (from 33.7 ± 2.4 to 36.6 ± 3.2 ml/kg/min, $p = 0.035$), forced vital capacity (from 3.5 ± 0.6 to 3.9 ± 0.9 l, $p = 0.044$), forced expiratory volume (from 3.2 ± 0.6 to 3.5 ± 0.7 l, $p = 0.014$), and peak expiratory flow (from 6.7 ± 1.4 to 7.5 ± 1.6 l/s, $p = 0.019$) also slightly improved in the omega-3 fatty acid group. Daily supplementation of 330 mg of omega-3 fatty acids had a slight positive impact on total cholesterol and glucose level, while there was no effect on low and high density lipoproteins, and triglycerides levels. Therefore, dose of 330 mg per day seems as insufficient.

Keywords: omega-3 fatty acids, vitamin D, blood lipids, glucose, anthropometric indices, VO₂max

Introduction

Fish oil omega-3 fatty acid supplementation can moderately reduce blood pressure [1], reduce the risk of cardiovascular disease and sudden cardiac death [2], and protect against cardiac arrhythmias [3]. However, changes in plasma lipid classes, especially after more than 3 months of supplementation, are not completely understood [4]. Some studies with a duration of 3–18 months have shown positive and significant lipid concentration changes in blood samples [5, 6], although each study used different supplements and a different ratio of active substances.

The use of omega-3 fatty acid food supplements is also associated with better physical performance – higher

omega-3 tissue levels may have a protective effect on muscle cells during exercise and reduce the inflammatory response and subsequent delayed onset muscle soreness [7]. It is recognized that regular consumption of polyunsaturated fatty acids or also can called polyunsaturated fatty acids (PUFAs) can improve lipid profiles, reduce oxidative stress, and reduce inflammation [8, 9]. Nevertheless, the correlation between blood glycemic levels and omega fatty acid consumption is not clear. In one study, no significant changes in this parameter were reported after consumption of fish oil for 3 months [10]. It is also known that the effects of dietary supplements are highly dependent on human anthropometric data, subjects' age, and daily physical activity level [11]. Moreover, because the dose of

supplementation appears to be important, it is not surprising that most studies on the impact of fish oil supplementation on human health parameters report conflicting results [12, 13, 14]. Nevertheless, the potential role of n-3 PUFAs in the maintenance of cardiovascular health and disease prevention has been highlighted [15]. The American Heart Association has set up dietary recommendations for EPA and DHA due to their cardiovascular benefits: individuals with no history of coronary heart disease or myocardial infarction should consume oily fish or fish oils two times per week; those having been diagnosed with coronary heart disease after infarction should consume 1 g EPA and DHA per day from oily fish or supplements; those wishing to lower blood triglycerides should consume 2–4 g of EPA and DHA per day in the form of supplements [16].

Both omega-3 and vitamin D supplements have also been increasingly used for the possible prevention of first cardiovascular event [17]. Because, the cardiometabolic disorders and vitamin D deficiency are more prevalent across multiple populations. Different studies have suggested a potential association between abnormal vitamin D levels and multiple pathological conditions including various cardiovascular diseases and diabetes [18]. There are several possible mechanisms contributing to the association between vitamin D and cardiovascular diseases, such as insulin sensitivity, parathyroid hormone elevation and inflammation [19]. The reason of this may be dyslipidemia, because dyslipidemia is a well-described independent risk factor for cardiovascular diseases [20].

Some results indicate that a vitamin D supplementation does not adversely affect weight loss and is able to improve several cardiovascular disease risk markers in overweight subjects with inadequate vitamin D status [21].

The purpose of this study was to test the effect of a daily dose of 330 mg of omega-3 fatty acid (sum of Docosahexaenoic Acid (DHA), Eicosapentaenoic Acid (EPA) and Docosapentaenoic acid (DPA)) with 0.005 mg (200 IU) of vitamin D3 as cholecalciferol on biochemical blood indices and aerobic capacity in healthy subjects compared with a daily dose of refined sunflower oil as placebo.

Methods

Study Protocol

Eighty-four healthy males and females volunteered to participate in this study. Written informed consent was received from each subject following a detailed explanation of the experimental protocol and any associated risks. Subjects were screened to ensure they were in good health, and were not using omega-3 fatty acids supplements and/or had

a diet rich in omega-3 fatty acids. Subjects were instructed to maintain their regular diet and exercise habits throughout the study period. Physical activity records were obtained only before the study; body composition parameters were obtained at the beginning, at the middle and at the end of the study period.

The inclusion criteria were as follows: male or female office workers aged 40–60 years, nonsmokers, and subjects with stable eating patterns and physical activity. The exclusion criteria included previous or current use of lipid-lowering therapies, treatment with vitamin D for osteoporosis, diabetes treated or detected at the inclusion visit, chronic diseases, and severe medical conditions that could interfere with the study, such as digestive tract surgery. Study participants' characteristics are presented in Table 1. The study protocol was approved by the Ethics Committee (2015-12-29, No BE-2-37, LUHS Kaunas Region Biomedical Research Ethics Committee, Lithuania).

An open, randomized, placebo-controlled, parallel-group study was conducted between January 2016 and September 2016 in Kaunas, Lithuania. The participants were first screened for eligibility through a phone call. All potential candidates underwent a prescreening visit during which the inclusion/exclusion criteria were detailed, and the study and the procedures to be followed were explained. The study coordinator answered any questions posed by the subjects, and their written informed consent was obtained. Thereafter, an anthropometric examination and the International Physical Activity Questionnaire were conducted, and blood samples were taken to confirm eligibility. Eligible subjects were randomly assigned to take either the placebo or the dietary supplement of omega-3 fatty acids in the morning hours, on empty stomach before breakfast. All participants were assessed at baseline (1T), at the middle (2T) (week 12), and at the end of the study (3T) (week 16) (Figure 1).

Anthropometrical measurement, spirometry and physical performance tests have been performed in the specialized laboratory of the Institute of Sport Science and Innovations, Lithuanian Sports University.

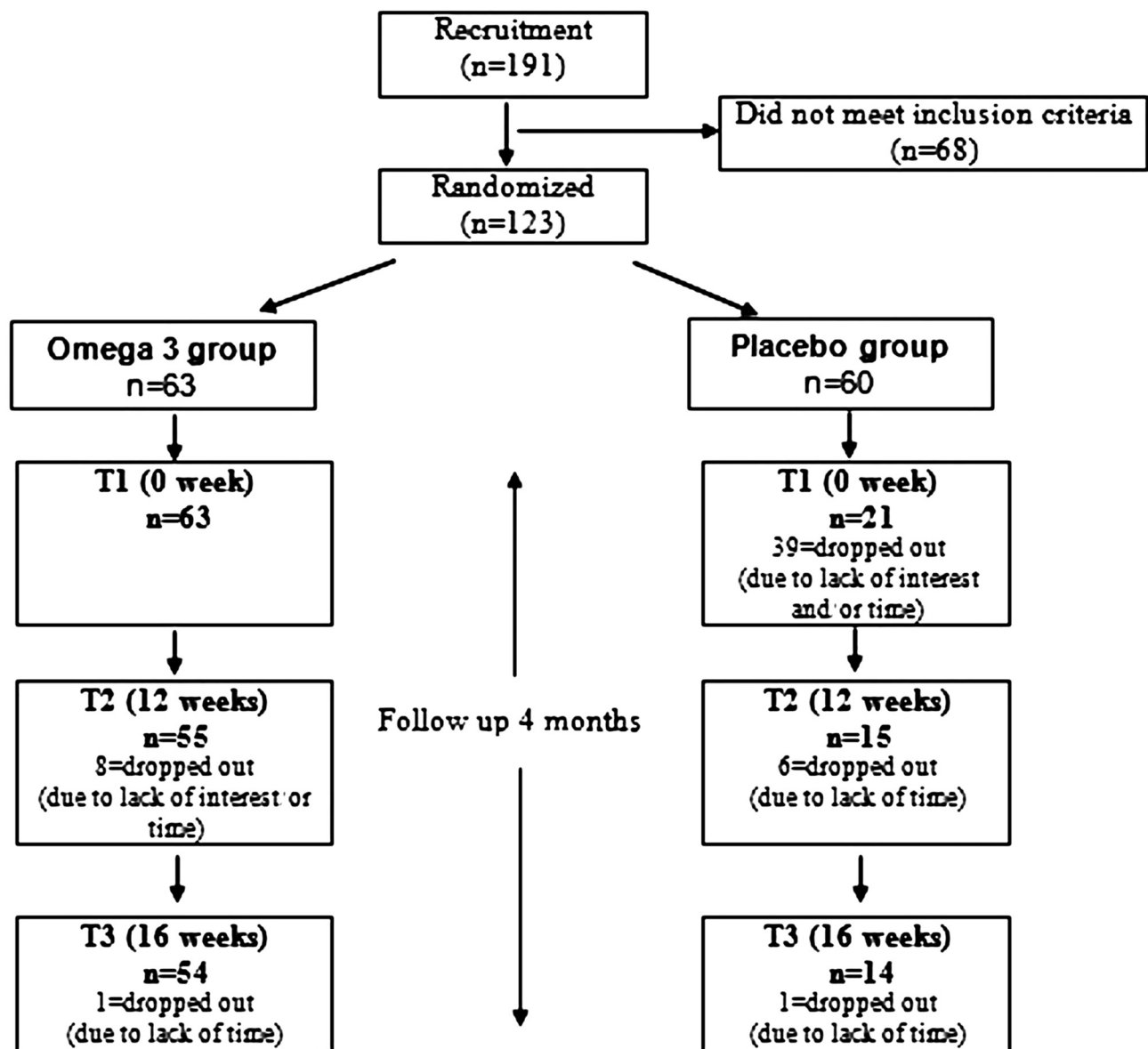
Supplements

Study participants received either a daily supplement of 330.2 mg omega-3 fatty acid (single dose: EPA – 91.5 mg, DHA – 63.0 mg, DPA – 10.6 mg) with 0.005 mg (200 IU) of vitamin D3 as cholecalciferol or 720 mg high-oleic sunflower oil as placebo for 16 weeks. The temperature of extraction was not specified. Supplements and placebo were produced by the company Pharmatech AS Product, Vallehellene 4, 1664 Rolvsøy, Norway.

Table 1. Characteristics of study participants.

	Omega 3 fatty acid (n = 63)	Placebo (n = 21)	p
Sex (n): Male	28	4	
Female	38	17	
Age (y)	44.3 ± 9	47.8 ± 9.8	.953
Height (m)	1.71 ± 0.06	1.69 ± 0.05	.118
Body mass (kg)	79.9 ± 18.7	73.6 ± 12.6	.141
Body mass index (kg/m ²)	27.5 ± 5.8	24.2 ± 3.7	.036
Fat mass (%)	32.2 ± 8.9	32.9 ± 11.2	.734
Physical activity (MET-min/wk)	2956.9 ± 2013.2	2835.04 ± 1263.3	.868
Education	High	High	

Notes: Values are expressed as mean ± sd.

**Figure 1.** Flowchart of the study participants.

Questionnaire

The short version of the International Physical Activity Questionnaire (IPAQ) was used to assess health-related physical activity.

Anthropometry

A body composition analyzer (Tanita, TBF-300, Illinois, USA) was used to measure body mass (BM, kg), body mass index (BMI, kg/m²), and fat mass (FM, %).

Physical Performance

Physical performance was measured using the Balke treadmill exercise test, which comprised a 4-min warm-up followed by an incremental continuous increase in incline of 2.5% every 2 min until volitional fatigue. The criteria used to verify that VO₂max was achieved were a respiratory exchange ratio greater than 1:1, maximum heart rate equal to 220 – age ± 10 beats per min, and a plateau in oxygen uptake with increasing workload. Pulmonary gas exchange was analyzed using a portable analyzer (Oxycon Mobile; Jaeger, Hoechberg, Germany). Before each test, the equipment was calibrated according to the manufacturer's recommendations.

Spirometry

The most common parameters of pulmonary function were measured using a portable gas analyzer (Oxycon Mobile, Jaeger): maximal oxygen consumption (VO₂max), forced vital capacity (FVC), forced expiratory volume (FEV1), peak expiratory flow (PEF), and maximum voluntary ventilation (MVV). Before each test, the analyzer was calibrated according to the manufacturer's recommendations.

Biochemical Analysis

Venous blood samples were drawn at the same time (±2 h) after an overnight fast (≥12 h) before supplementation, and after 12 and 16 weeks. Plasma vitamin D3 ("COBAS 6000 e601"), glucose level, and blood lipid profile ("COBAS INTEGRA 400 plus")—total cholesterol (TCh), high-density cholesterol (HDL), low-density cholesterol (LDL), and triglycerides (TG)—were assessed. All biochemical analyses have been carried out in the leading private hospital in Lithuania "Kardiolita Hospital. BMP", company code:133643318, VAT No. LT336433113, Address: Savanorių pr. 423, Kaunas, Lithuania, LT-49287

Statistical Analysis

The data were tested for normal distribution using the Kolmogorov-Smirnov test, and all data were found to be normally distributed. Two-way mixed analysis ANOVA (General Linear Model) was used to determine the effect of the repeated measurements as within-subject factor of tree levels and groups – Omega-3 fatty acid and placebo as between subject factor on the BM, BMI, FM, glucose, TCh, HDL, LDL, TG, vitamin D, VO₂max, FVC, FEV1, PEF, and MVV. A significant result was followed by LSD post hoc adjustment to determine differences among measurement conditions. If Mauchly's Test of Sphericity was significant then the Greenhouse-Geisser correction was used. The level of significance was set at $p < 0.05$ and effect size (partial eta squared) as well as observed power were also calculated and reported. All statistical analyses were performed using IBM SPSS Statistics 22 (IBM Corporation, Armonk, NY).

Results

FM did not change in either group over the 16-week supplementation period; however, BM after 16 weeks (3T) of supplementation significantly decreased by 1.6% from 74.4 ± 13.04 to 73.2 ± 13.02 kg ($p = 0.000$) in the omega-3 fatty acid group, and by 1.2% from 69.5 ± 11.1 to 68.7 ± 11.4 kg ($p = 0.047$) kg in the placebo group. BMI also significantly decreased after 16 weeks (3T) by 1.6% from 25.8 ± 4.1 to 25.4 ± 4.3 kg/m² ($p = 0.000$) in the omega-3 fatty acid group and by 1.2% from 24.1 ± 4 to 23.8 ± 4.2 kg/m² in the placebo group ($p = 0.048$) (Table 2).

In the omega-3 fatty acid group, 16 weeks of supplementation (3T) significantly increased vitamin D concentration by 47.8% from 35.07 ± 21.65 to 67.15 ± 22.93 nmol/l; glucose significantly decreased by 2.9% after 12 weeks (2T) of supplementation ($p = 0.005$) from 5.12 ± 0.55 to 4.97 ± 0.62 mmol/l, but it remained unchanged at 3T. The TCh level at 3T decreased by 9.2% compared with the baseline level (1T) from 5.86 ± 1.0 to 5.32 ± 1.55 mmol/l ($p = 0.003$). The HDL, LDL, and TG parameters did not change significantly within the study period (Table 3).

In the placebo group, only vitamin D concentration increased significantly by 63.4% from 25.62 ± 13.85 to 70.05 ± 16.94 nmol/l by 3T ($p = 0.000$); other blood parameters were unchanged.

The increment in the relative VO₂max value (Fig. 2) was measured in the omega-3 fatty acid group at 3T comparing with the baseline at 1T (from 33.7 ± 2.4 to 36.6 ± 3.2 ml/min/kg, $p = 0.035$) and at 3T compared with the second testing (2T) (from 33.6 ± 2.5 to 36.6 ± 3.2 ml/min/kg, $p = 0.023$). No significant differences were found in the placebo group.

Table 2. Effects of omega-3 fatty acid and placebo on subjects' body composition.

Indexes	Omega-3 fatty acids			p			Observed Power
	1T (n = 63)	2T (n = 55)	3T (n = 54)	1/2	1/3	2/3	
Body mass (kg)	74.4 ± 13.0	73.3 ± 13.2	73.2 ± 12.0	.000	.000	.265	1.000
Body mass index (kg/m ²)	25.8 ± 4.1	25.5 ± 4.3	25.4 ± 4.3	.000	.000	.262	1.000
Fat mass (%)	30.4 ± 8.0	30.1 ± 8.2	29.9 ± 8.2	.178	.097	.347	.422
	Placebo			p			
	1T (n = 21)	2T (n = 15)	3T (n = 14)	1/2	1/3	2/3	
Body mass (kg)	69.5 ± 11.1	68.4 ± 11.6	68.7 ± 11.4	.024	.047	.053	0.637
Body mass index (kg/m ²)	24.1 ± 4	23.8 ± 4.3	23.8 ± 4.2	.045	.048	.136	0.519
Fat mass (%)	30.4 ± 2.6	30.3 ± 2.7	30.7 ± 2.6	.871	.442	.202	0.151

Notes: Values are expressed as mean ± sd.

1T, before the study; 2T, at the middle of the study; 3T, at the end of the study.

1/2 – statistical difference between 1T and 2T; 1/3 – statistical difference between 1T and 3T; 2/3 – statistical difference between 2T and 3T.

Table 3. Effects of omega-3 fatty acid and placebo on biochemical blood indices.

Indexes	Omega-3 fatty acids			p			Observed Power
	1T (n = 63)	2T (n = 55)	3T (n = 54)	1/2	1/3	2/3	
Glucose (mmol/l)	5.12 ± 0.6	4.97 ± 0.62	5.12 ± 0.77	.005	.450	.138	0.583
Cholesterol (mmol/l)	5.86 ± 1.0	5.73 ± 1.06	5.32 ± 1.55	.090	.003	.025	0.825
HDL (mmol/l)	1.99 ± 0.52	1.99 ± 0.52	2.06 ± 0.76	.539	.392	.345	0.145
LDL (mmol/l)	3.26 ± 0.89	3.24 ± 0.86	3.18 ± 1.25	.850	.528	.567	0.093
TG (mmol/l)	1.38 ± 0.94	1.29 ± 0.68	1.23 ± 0.56	.226	.068	.373	0.426
Vitamin D (nmol/l)	35.07 ± 21.65	68.63 ± 25.94	67.15 ± 22.93	.000	.000	.659	1.000
	Placebo			p			
	1T (n = 21)	2T (n = 15)	3T (n = 14)	1/2	1/3	2/3	
Glucose (mmol/l)	5.08 ± 0.6	4.94 ± 0.74	5.02 ± 0.99	.473	.807	.733	0.078
Cholesterol (mmol/l)	5.45 ± 1.12	5.45 ± 1.18	4.78 ± 1.43	.987	.116	.100	0.372
HDL (mmol/l)	1.99 ± 0.57	1.91 ± 0.54	2.0 ± 0.83	.219	.713	.484	0.113
LDL (mmol/l)	2.95 ± 0.77	3.02 ± 0.89	2.74 ± 0.88	.579	.435	.392	0.171
TG (mmol/l)	1.73 ± 0.40	1.11 ± 0.47	1.30 ± 1.07	.910	.517	.536	0.106
Vitamin D (nmol/l)	25.62 ± 13.85	80.66 ± 27.70	70.05 ± 16.94	.000	.000	.074	1.000

Notes: Values are expressed as mean ± sd.

1T, before the study; 2T, at the middle of the study; 3T, at the end of the study.

1/2 – statistical difference between 1T and 2T; 1/3 – statistical difference between 1T and 3T; 2/3 – statistical difference between 2T and 3T.

HDL – high density cholesterol; LDL – low density cholesterol; TG – triglycerides.

FVC (Fig. 3) was significantly higher after 12 weeks (2T) (from 3.5 ± 0.6 to 3.8 ± 0.8 l, $p = 0.047$) and 16 weeks (3T) (from 3.5 ± 0.6 to 3.9 ± 0.9 l, $p = 0.044$) compared with the baseline at 1T in the omega-3 group. There were no significant differences in the placebo group.

Figure 4 shows that 16 weeks (3T) of supplementation significantly increased FEV1 in the omega-3 fatty acid group (from 3.2 ± 0.6 to 3.5 ± 0.7 l, $p = 0.014$) compared with the beginning at 1T, but no significant differences were found between the two groups.

PEF was significantly higher (Fig. 5) after 16 weeks (3T) of supplementation in the omega-3 fatty acid group (from 6.7 ± 1.4 to 7.5 ± 1.6 l/min, $p = 0.019$) comparing with the baseline at 1T, but no significant differences were found in the

placebo group. MVV did not significantly change over the 16 weeks in either group.

Discussion

The anthropometric results indicate that body weight and body mass index decreased in the omega-3 fatty acid group after 16 weeks of supplementation. Numerous studies using a fish oil dose of 1–1.5 g and a duration of use of up to 24 weeks have evaluated the effect of fish oil supplementation on body composition. Most of these studies demonstrated a positive impact on body weight and body mass index after

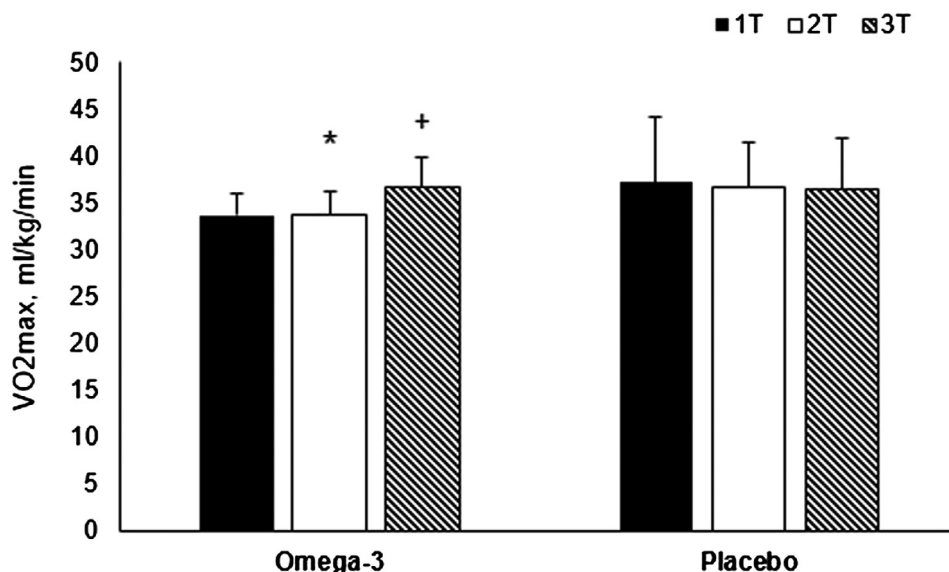


Figure 2. Effects of omega-3 fatty acid and placebo on maximal oxygen consumption. Notes: 1T, before the study (omega 3 fatty acid group n = 63; placebo n = 21); 2T, at the middle of the study (omega 3 fatty acid group n = 55; placebo n = 15); 3T, at the end of the study (omega 3 fatty acid group n = 54; placebo n = 14). *Significantly different between 1T and 3T (1/3 p = 0.035). +Significantly different between 2T and 3T (2/3 p = 0.023). Error bar represent $\pm$ standard deviation.

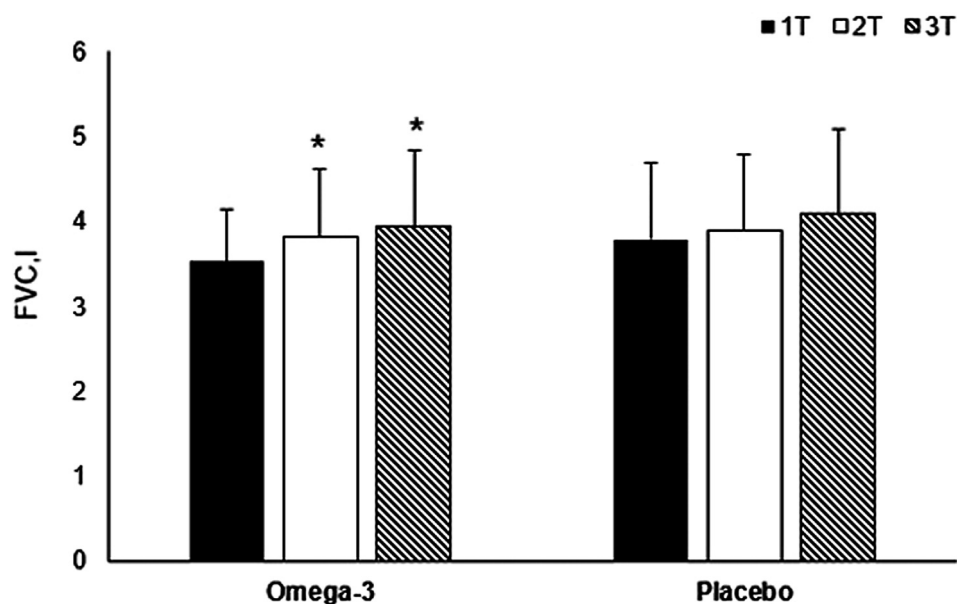


Figure 3. Effects of omega-3 fatty acid and placebo on forced vital capacity. Notes: 1T, before the study (omega 3 fatty acid group n = 63; placebo n = 21); 2T, at the middle of the study (omega 3 fatty acid group n = 55; placebo n = 15); 3T, at the end of the study (omega 3 fatty acid group n = 54; placebo n = 14). *Significantly different between 1T and 2T (1/2 p = 0.041) and between 1T and 3T (1/3 p = 0.042). Error bar represent $\pm$ standard deviation

omega-3 fatty acid supplementation [22, 23, 24, 25, 26, 27]. The results from our study are in line with these findings. Several studies have reported a reduction in body fat mass [24, 28, 29, 30, 31]. However, our results did not show a significant decrease of fat mass in either group, even though the duration of fish oil supplementation was similar (6–16 weeks) but the dose much larger (1.8–6 g) in those studies.

Table 5 summarizes body composition index results obtained after consumption of different doses and duration of fish oil.

Fish oil appears to have multiple mechanisms of action in the body and can provide benefits to cardiovascular health. The 2002 American Heart Association Scientific Statement published after landmark trials highlighted the benefit of

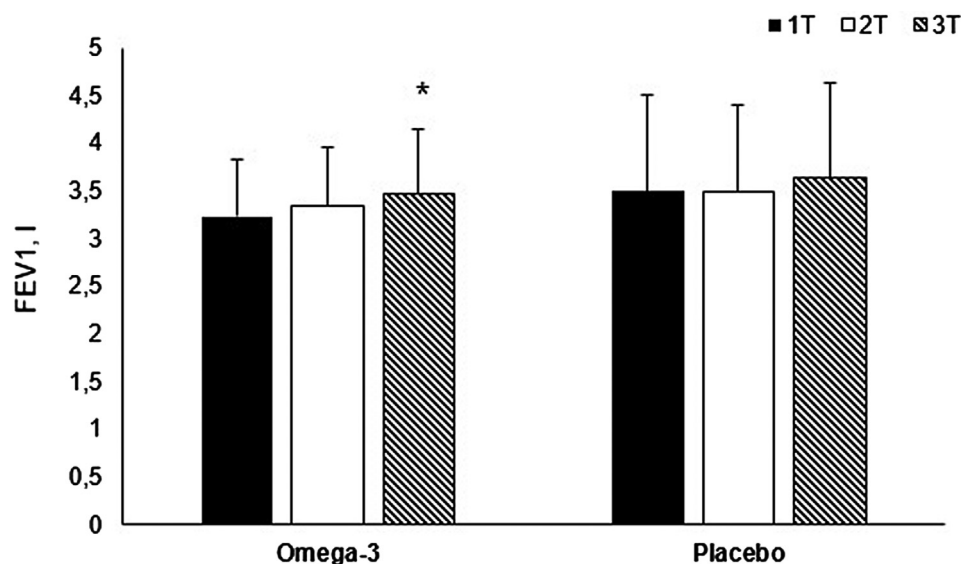


Figure 4. Effects of omega-3 fatty acid and placebo on forced expiratory volume. Notes: 1T, before the study (omega 3 fatty acid group n = 63; placebo n = 21); 2T, at the middle of the study (omega 3 fatty acid group n = 55; placebo n = 15); 3T, at the end of the study (omega 3 fatty acid group n = 54; placebo n = 14). *Significantly different between 1T and 3T (1/3 p = 0.034). Error bar represent ± standard deviation

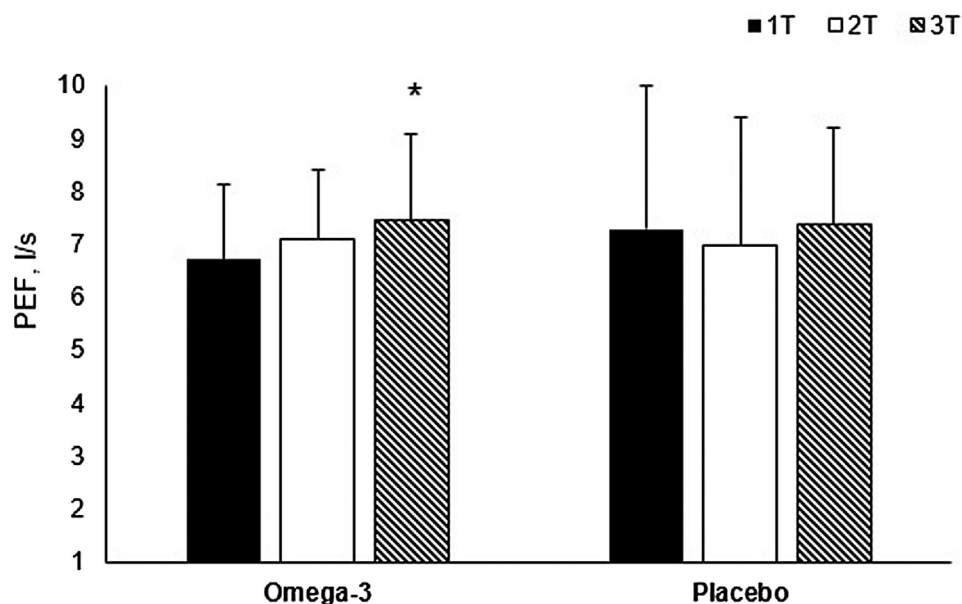


Figure 5. Effects of omega-3 fatty acid and placebo on peak expiratory flow. Notes: 1T, before the study (omega 3 fatty acid group n = 63; placebo n = 21); 2T, at the middle of the study (omega 3 fatty acid group n = 55; placebo n = 15); 3T, at the end of the study (omega 3 fatty acid group n = 54; placebo n = 14). *Significantly different between 1T and 3T (1/3 p = 0.028). Error bar represent ± standard deviation

fish in reducing morbidity and mortality in those with cardiovascular disease. Since then, numerous studies have shown the benefit of fish oil in decreasing TG levels, promoting antiplatelet activity, decreasing heart failure, and improving vascular function in diabetes. Fish oil was shown to improve TG levels in combination with other lipid-lowering therapies such as statins and fibrates, and was also reported to have an effect on lowering levels of TGs and VLDL and increasing HDL [32]. Systematic reviews have

shown that individuals with any degree of dyslipidemia, and elevated serum TG and/or cholesterol levels may benefit from a 20–30% reduction in serum TG after consuming n-3 PUFAs derived from marine sources [15].

Fish oil is popularly used for reducing TGs or improving dyslipidemia. It was shown that fish oil supplementation produces a clinically significant dose-dependent reduction of fasting blood TG, but not total, HDL, or LDL cholesterol in hyperlipidemia subjects [32]. A reduction of 9–26% in

Table 4. Effects of omega-3 fatty acid and placebo on spirometric indices.

Indexes	Omega-3 fatty acids			p			Observed Power
	1T (n = 63)	2T (n = 55)	3T (n = 54)	1/2	1/3	2/3	
VO ₂ max (ml/kg/min)	33.7 ± 2.4	33.66 ± 2.5	36.6 ± 3.2	.931	.035	.023	0.864
FVC (l)	3.5 ± 0.6	3.8 ± 0.8	3.9 ± 0.9	.047	.044	.203	0.765
FEV1 (l)	3.2 ± 0.6	3.4 ± 0.6	3.5 ± 0.7	.072	.014	.105	0.866
PEF (l/s)	6.7 ± 1.4	7.1 ± 1.3	7.5 ± 1.6	.107	.019	.079	0.827
MVV (l/min)	124.8 ± 33.1	130.6 ± 32	133.4 ± 34.6	.224	.294	.653	0.192
	Placebo			p			
	1T (n = 21)	2T (n = 15)	3T (n = 14)	1/2	1/3	2/3	
VO ₂ max (ml/kg/min)	37.2 ± 11.3	36.6 ± 7.1	36.4 ± 5.4	.838	.838	.855	0.054
FVC (l)	3.8 ± 0.9	3.9 ± 0.9	4.1 ± 1.0	.471	.084	.473	0.244
FEV1 (l)	3.5 ± 1	3.5 ± 0.9	3.7 ± .0	.100	.074	.074	0.218
PEF (l/s)	7.3 ± 2.7	7.0 ± 2.4	7.4 ± 1.9	.149	.912	.427	0.098
MVV (l/min)	125.73 ± 25.9	130.6 ± 30.0	139.8 ± 27.4	.587	.109	.175	0.417

Notes: Values are expressed as mean ± sd.

1T, before the study; 2T, at the middle of the study; 3T, at the end of the study.

1/2 – statistical difference between 1T and 2T; 1/3 – statistical difference between 1T and 3T; 2/3 – statistical difference between 2T and 3T.

VO₂max – maximal oxygen consumption; FVC – forced vital capacity; FEV1 – forced expiratory volume; PEF – peak expiratory flow; MVV – maximum voluntary ventilation.

circulating TGs was demonstrated in studies where ≥ 4 g/day n-3 PUFAs were consumed from either marine or EPA/DHA-enriched food sources. A reduction of 4–51% was found in studies where 1–5 g/day of EPA and/or DHA was consumed through the supplements [15].

On the contrary, our results showed that TG levels did not change significantly in the omega-3 fatty acid group, which was likely due to the low dose of supplementation. Poppitt et al. [34] also found that even 3 g/day of encapsulated fish oil (containing 1.2 g/day total omega-3 PUFAs) used for 12 weeks had no significant effect on TG levels. However, as seen in Table 5, other studies that used different doses and durations of supplementation showed opposite effects.

Other studies have reported that daily supplementation of 720 mg of omega-3 fatty acid had no effect on LDL and HDL levels. Our results confirm the findings of other studies [13, 34, 35, 36, 37, 38] that showed no significant effect on blood lipid fractions. In those studies, the duration of supplementation varied from 6 to 12 weeks and the dose used varied from 0.85 g to 4.4 g.

Our results showed that serum glucose and TCh levels were significantly reduced in the omega-3 fatty acid group. However, in general, other studies [35, 39] reported no effect on these blood induces levels [33, 36, 37, 40]. In contrast, Mostad et al. [41] reported that a high intake of fish oil (17.6 ml/day) used for 9 weeks was able to increase blood glucose level.

It is known that the effects of dietary supplements are highly dependent on human anthropometric data, subjects' age, and daily physical activity level [11]. Moreover, the dose of supplementation seems to be very important. In our case, because the supplementation dose was for

preventive use only, it may have been too low to induce notable blood lipid changes. Different global organizations recommend varying daily doses of EPA/DHA. However, most experts recommend that adults should consume at least 500 mg of EPA/DHA daily to maintain good health [42, 43]. Table 6 summarizes the main biochemical blood and respiratory index results, and other findings obtained from adults after consumption of different doses and duration of fish oil.

In our study, vitamin D status significantly increased in both groups after 16 weeks of supplementation. However, we believe that this was not as a result of low dose 200 IU of vitamin D supplementation, because the second part of study was conducted in summertime and changes in vitamin D levels were likely related to greater sun exposure. Numerous studies (Table 7) have reported on the effects of seasonal changes on vitamin D status in countries located in northern latitudes [44, 45, 46, 47, 48, 49, 50]; our results confirm these effects.

The relation of Vitamin D and glucose metabolism and lipid profile is very inconsistent in the literature. Gupta et al. [51] reported that low vitamin D levels increased pre-diabetes risk, and in healthy adolescent males, the insulin levels decreased as the vitamin D level increased. Pittas et al., [52] reported that the type 2 diabetes risk and vitamin D insufficient is very close. The higher 25(OH) D level, the lower type 2 diabetes risk [52]. But in a study carried out with postmenopausal women [53], it was reported that low vitamin D levels were not associated with diabetes risk. In our case, after the middle testing (2T) glucose level significantly decreased ($p < 0.05$), and vitamin D level significantly increased at the same time. We did not evaluate insulin

Table 5. Studies assessing body composition outcome effects on Omega-3 fatty acids by dietary intervention.

Study	Subject characteristics	Omega-3 Source (dose/day)	Duration	Body composition outcomes	Other findings
Krebs et al., 2006 [19]	116 overweight insulin-resistant women	Control group (no weight-loss and placebo oil) Weight-loss intervention groups with either supplemental LC n-3 PUFA (WLFO) or placebo oil (WLPO). WLFO group received five 1 g oil capsules per day with predominantly LC n-3 PUFA, totalling 1.3 g EPA and 2.9 g DHA. WLPO and control group received five 1 g oil capsules per day, containing 2.8 g linoleic acid and 1.4 g oleic acid.	24 weeks	Significant weight-loss was in WLFO (10.8 ± 1.0%) and WLPO (12.4 ± 1.0%) compared to the control group. The WLFO, but not WLPO or control group, showed significant increases in adipose tissue LC n-3 PUFA.	Significant decreases in triglycerides and increases in adiponectin was registered with LC n-3 PUFA, in the WLFO vs WLPO groups.
Kunesova et al., 2006 [18]	20 obese woman	n-3 PUFA and placebo; Plus diet – 2200 kJ/day 60 min. light or middle physical activity/day	3 weeks	The addition of n-3 PUFA of fish origin to a very low calorie diet results in a greater BMI loss and hip circumference reduction.	
Hill et al., 2007 [25]	Overweight subjects, aged 25–65 y.	Fish oil (FO); FO and exercise (FOX); Sunflower oil (SO; control); SO and exercise (SOX)	12 weeks	Both fish oil and exercise independently reduced body fat.	FO supplementation lowered TG, increased HDL cholesterol, and improved endothelium-dependent arterial vasodilation.
Kabir et al., 2007 [24]	27 women with type 2 diabetes without hypertriglyceridemia	(1) 3 g/d of either fish oil (containing 1.8 g n-3 PUFAs – 1.08 g EPA and 0.72 g DHE) (2) placebo (paraffin oil)	2 months	Body weight were unchanged, total fat mass and subcutaneous adipocyte diameter significantly reduced in the fish oil group.	Plasma TG and plasma plasminogen activator inhibitor-1 were significantly reduced in the fish oil group.
Thorsdottir et al., 2007 [20]	324 men and woman, overweight, aged 20–40 years	(1) control-sunflower oil capsules, no seafood; (2) lean fish – 3–150 g portions of cod/week; (3) fatty fish – 3–150 g portions of salmon/week; (4) fish oil-DHA/EPA capsules, no seafood	8 weeks	In young, overweight men, the inclusion of either lean or fatty fish, or fish oil as part of a hypoenergetic diet resulted in 1 kg more weight-loss after 4 weeks than did a similar diet without seafood or supplement of marine origin.	
Bays et al., 2009 [54]	167 dyslipidemic, overweight/obese patients aged 18 to 79 years.	OM3 4 g/day + fenofibrate 130 mg/day (n = 84) versus PLACEBO (4 g/day of corn oil) + fenofibrate 130 mg/day (n = 83), and an 8-week open-label extension (n = 117), during which all subjects received P-OM3 + fenofibrate. Subjects who received P-OM3 + fenofibrate continued the same treatment in the extension phase (non-switchers; n = 59). Those who initially received corn oil placebo + fenofibrate received P-OM3 + fenofibrate in the extension phase (switchers; n = 58)	16 weeks	No effect on body weight or waist circumference noted	

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Table 5. (Continued)

Study	Subject characteristics	Omega-3 Source (dose/day)	Duration	Body composition outcomes	Other findings
Noreen et al., 2010 [26]	44 men and women, aged 34–45 years.	4 g/d of Safflower Oil (SO); 4 g/d of fish oil (FO)	6 weeks	FO significantly increased lean mass and decreased fat mass.	
DeFina et al., 2011 [55]	128 overweight and obese individuals, aged 30–60 years.	(1) 5 omega-3 capsules/day (3.0 g EPA plus DHA at a 5:1 ratio EPA:DHA) (2) 5 placebo capsules/day	24 weeks	No significant weight-reduction benefit was seen with the addition of omega-3 fatty acid supplementation.	
Crochemore et al., 2012 [21]	41 women (60.64 ± 7.82 years) with high blood pressure and diabetes mellitus	3 groups: GA (2.5 g/d fish oil), GB (1.5 g/d fish oil), GC (control)	30 days	GB presented a greater loss of body mass and waist circumference, compared with GA.	GB presented a greater frequency of glycemic and total cholesterol reduction, and an increase of high-density lipoprotein cholesterol compared with GA.
Munro & Garg, 2012 [27]	Obese subjects, aged 18–60	(1) Placebo (PB) – 6 × 1 g capsules/d of monounsaturated oil; (2) Fish oil (FO) 6 × 1 g capsules/d of LCn-3PUFA	16 weeks	There was a significant reduction in fat mass for the FO group at week 14 but not for PB.	Both groups experienced improved metabolic profiles
Harden et al., 2014 [22]	Overweight and obese woman	DHA and placebo	12 weeks	Body weight decreased in the DHA group.	
Huerta et al., 2015 [23]	97 overweight and obese woman	(1) Control; (2) EPA (1.3 g/d); (3) α-lipoic acid (0.3 g/d); (4) EPA + α-lipoic acid (1.3 g/d + 0.3 g/d)	10 weeks	Body weight loss was significantly higher in those groups supplemented with α-lipoic acid.	EPA supplementation significantly attenuated the decrease in leptin levels that occurs during weight loss. Body weight loss improved lipid and glucose metabolism parameters but without significant differences between groups.

level. So, we cannot confirm that elevated vitamin D level affected glucose level.

Levels of serum cholesterol are a strong predictor of cardiovascular risk. Observational studies have demonstrated that high levels of vitamin D are associated with a favorable lipid profile, whereas low levels of vitamin D are associated with an atherogenic lipid profile [54]. Our results showed that serum TCh level was significantly reduced in the omega-3 fatty acid group, but omega-3 fatty acids and vitamin D supplement had no effect on other blood lipid parameters for the omega 3 group and placebo group.

Supplementation of omega-3 fatty acid improved VO₂max outcome and some respiratory function indices compared with the placebo group. Haghravan et al. [55] studied the effect of omega-3 supplementation with lifestyle

modification on VO₂max in overweight women. They reported that those individuals who followed an aerobic exercise program and received fish oil supplement significantly increased oxygen consumption. Our results agree with their findings, although our participants did not follow a specific aerobic exercise program. There are few reports on the direct effects of omega-3 fatty acid supplementation on physical performance. Brilla et al. [56] reported that those sedentary males who were supplemented with fish oil (4 g/day) for 10 weeks with exercise three times a week showed no additional effect on VO₂max compared with those who exercised only without fish oil supplementation.

It has been reported that fish oil can enhance muscle and maximum oxygen uptake (VO₂max), thereby improving endurance performance recovery [57] and cardiovascular

Table 6. Studies assessing biochemical blood outcome effects of Omega-3 fatty acid by dietary intervention.

Study	Subject characteristics	Omega-3 fatty acids Source (dose/day)	Duration	Blood biochemical outcomes	Other findings
Borkman et al., 1989 [56]	10 subjects (aged 42–65 yr) with type 2 diabetes	(1) 10 g fish oil concentrate (30% omega 3FAs) daily (2) 10 g safflower oil daily	3 weeks	Fasting blood glucose increased 14% during fish oil and 11% during safflower oil supplementation compared with baseline, whereas fasting serum insulin levels, and insulin sensitivity were unchanged	Body weight were unchanged
Woodman et al., 2002 [31]	59 subjects, aged 40–75 y with type 2 diabetes	(1) 4 g EPA/d (2) 4 g DHA/d, (3) olive oil/d – placebo	6 weeks	Neither EPA nor DHA had significant effects on glycated hemoglobin, fasting insulin or C-peptide. Serum TG in the EPA and DHA groups decreased 19% and 15%. There were no significant changes in serum total, LDL, or HDL cholesterol, although HDL-cholesterol (2) in the EPA and DHA groups increased 16% and 12%. HDL(3) cholesterol decreased 11% (P = 0.026) with EPA supplementation.	Neither EPA nor DHA had significant effects on fasting insulin or C-peptide, insulin sensitivity or secretion, or blood pressure.
Ciubotaru et al., 2003 [57]	30 healthy subjects	14 g/day safflower oil (SO); 7 g/day of both safflower oil and fish oil (LFO); 14 g/day fish oil (HFO)	5 weeks	Plasma (TG) decreased in the HFO compared to the SO group.	CRP decreased and IL-6 compared to SO, with a greater effect in the LFO than HFO groups.
Mostad et al., 2006 [37]	26 subjects with type 2 diabetes without hypertriacylglycerolemia	Intervention group was 17.6 mL fish oil/d (1.8 g 20:5n ₃ , 3.0 g 22:6n ₃ , and 5.9 g total n ₃ fatty acids). The control gr. received 17.8 mL corn oil/d (8.5 g 18:2n ₆).	1 week and 9 wks	A high intake of fish oil moderately increases blood glucose.	A high intake of fish oil decreases insulin sensitivity in persons with type 2 diabetes and alters carbohydrate and fat utilization in a time-dependent manner.
Cazzola et al., 2007 [32]	Healthy young (18–42 years) and older (53–70 years) men.	Placebo or 1.35, 2.7 or 4.05 g EPA/day.	12 weeks	No effect on on plasma total, LDL or HDL cholesterol. EPA lowered plasma TG, with the max effect at the lowest dose.	-
Damsgaard et al., 2008 [35]	Healthy men (n = 64), aged 19–40.	(1) 5 mL/d fish oil capsules (FO) (Bio-marine, FFA), mean intake 3.1 g/d (n-3) LCPUFA. (2) 5 mL/d Olive oil (OO) (unrefined extra virgin, TAG) capsules (control). Within each group, they were also allocated to use fats either with a high (S/B) or a low (R/K) LA content, resulting in a 7.3 g/d higher LA intake in the S/B groups than in the R/K groups.	8 weeks	FO lowered fasting plasma TAG by 51% and 19% in the FO R/K-group and FO S/B-group, respectively. Neither the FO nor fat intervention affected fasting plasma cholesterol, glucose level.	Neither the FO nor fat intervention affected insulin, fibrinogen, C-reactive protein, interleukin-6, vascular cell adhesion molecule-1, P-selectin, oxidized LDL, cluster of differentiation antigen 40 ligand (CD40L), adiponectin, or fasting or postprandial BP or HR after adjustment for body weight changes.

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Table 6. (Continued)

Study	Subject characteristics	Omega-3 fatty acids Source (dose/day)	Duration	Blood biochemical outcomes	Other findings
Egert et al., 2009 [33]	74 healthy normolipidemic men and women aged 19–43 y	ALA group – 4.4 g/d; EPA group – 2.2 g/d EPA; DHA group – 2.3 g/d DHA	6 weeks	ALA, EPA, or DHA intake did not affect fasting serum concentrations of total and LDL cholesterol, but fasting serum TG significantly decreased in the EPA (20.14 mmol/L) and DHA (20.30 mmol/L) and also in the ALA intervention (20.17 mmol/L). DHA intake significantly increased serum HDL cholesterol, whereas no changes were found with ALA or EPA intake.	
Eslick et al., 2009 [29]	47 participants	(1) 3.25 g of EPA and/or DHA daily (2) Placebo		Fish oil supplementation reduced TG (–0.34 mmol/L), no change in total cholesterol (–0.01 mmol/L) and very slight increases in HDL (0.01 mmol/L) and LDL cholesterol (0.06 mmol/L).	
Poppitt et al., 2009 [30]	102 participants, aged > 45 years.	(1) 3 g/day encapsulated fish oil (containing 1.2 g/day total omega-3 PUFA) (2) Placebo	12 weeks	No significant effect of fish oil treatment on TG, LDL-ch.	No significant effect of fish oil treatment on any cardiovascular markers, inflammatory and hemostatic parameters, blood pressure, health-related quality of life, or mood.
Shidfar et al., 2008 [36]	50 type 2 diabetes patients	2 g/day purified omega-3 FA or placebo	10 weeks	Fasting TG decreased significantly with supplementation relative to placebo. Omega-3 fatty acids had no significant effect on serum lipid levels, ApoA-I, glucose, insulin and HbA1c.	
Thusgaard et al., 2009 [34]	51 participant	(1) n-3 PUFA group – 2 capsules of Omacor twice daily; (2) 2 capsules of placebo	12 weeks	Plasma TG reduced in the n-3 PUFA group by 0.14 mmol/L, while TG increased by 0.36 mmol/L in the control group. No significant effect of treatment was found for total cholesterol, HDL or LDL-cholesterol or apolipoproteins.	
Dawczynski et al., 2010 [58]	51 adults	Both groups received intervention (3 g n-3 LC-PUFA/d) and control dairy products consecutively.	15 weeks	HDL decreased to a lesser extent than placebo, suggesting a relative increase.	
Fakhrzadeh et al., 2010 [59]	124 elderly healthy people, aged > or = 65	(1) intervention group – 1 g/day fish oil capsule (with 180 mg EPA and 120 mg DHA); (2) placebo group	6 months	In the placebo group, serum TG significantly increased and HDL-cholesterol decreased.	

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Table 6. (Continued)

Study	Subject characteristics	Omega-3 fatty acids Source (dose/day)	Duration	Blood biochemical outcomes	Other findings
Dewel et al., 2011 [60]	100 middle-aged adults (50 ± 10 yrs.) with metabolic syndrome	(1) low-dose flaxseed oil (LFX) (Barlean's Organic Oils) 2.2 g ALA/d, 4 capsules/d; (2) high-dose flaxseed oil (HFX) 6.6 g ALA/d, 12 capsules/d; (3) low-dose fish oil (LFO) (Nordic Naturals) 1.2 g EPA + DHA (700 mg EPA and 500 mg DHA)/d, 2 capsule/d; (4) high-dose fish oil (HFO) 3.6 g EPA + DHA (2.1 g EPA and 1.5 g DHA)/d, 6 capsules/d; (5) placebo (P) 4 or 6 g soybean oil/d, 4 or 6 capsules/d.	8 weeks	LDL-cholesterol increased in both fish oil groups compared to both flaxseed oil groups; TG decreased in the HFO group compared to both flaxseed oil groups; group compared to all other groups (P # 0.02).	There were no significant differences in changes in plasma inflammatory markers among groups in the subset with concentrations. Systolic blood pressure decreased in the HFO group compared to the HFX and P groups; and diastolic blood pressure decreased in the HFO
Maki et al., 2011 [61]	31 adults	Omega-3-acid ethyl esters (POM3, 4 g/day) versus placebo (soy oil).	6 weeks	Significant for POM3 were observed for VLDL- cholesterol (−18.8%), TG (−18.7%), and HDL-C (3.3%). Total cholesterol, non-HDL-C, apolipoproteins A1 and B, and LDL particle concentration responses did not differ between treatments.	
Skulas-Ray et al., 2011 [15]	26 (23 men and 3 postmenopausal women) 21–65 years age healthy persons with moderate hypertriglyceridemia	0 g EPA + DHA/d (corn oil placebo), 0.85 g EPA + DHA/d, and 3.4 g EPA + DHA/d.	8 weeks	TG reduced (27%) with the 3.4-g/d dose of EPA + DHA. Total cholesterol, LDL- cholesterol, and HDL- cholesterol values did not differ significantly by treatment. The lower dose (0.85 g/d) did not alter lipid values, and fasting measures of glucose metabolism were not altered by either dose relative to placebo.	No effects of 0.85 or 3.4 g EPA + DHA/d on endothelial function, insulin, glucose, or inflammation relative to placebo were observed. Liver enzymes (alanine aminotransferase and aspartate aminotransferase) and body weight (or BMI) were also unchanged.
Vargas et al., 2011 [62]	51 women, 20–45 y age with polycystic ovary syndrome.	3.5 g of total n-3 PUFA daily (essential PUFA from flaxseed oil or long-chain PUFA from fish oil) and soybean oil (placebo).	6 weeks	Fish oil and flaxseed oil lowered serum triglyceride (within-group after vs before intervention).	Weight, BMI, fat mass and waist circumference, fasting glucose, insulin, adiponectin, leptin, or high- sensitivity C-reactive protein did not change with any intervention.
Schirmer et al., 2012 [63]	53 participants	4 g n3-FA daily and placebo	3 weeks	n3-FA reduced fasting TG (18%) and postprandial TG (16%), while relative TG increase (192.8 ± 12.7%) was comparable to placebo.	

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Table 6. (Continued)

Study	Subject characteristics	Omega-3 fatty acids Source (dose/day)	Duration	Blood biochemical outcomes	Other findings
Oelrich et al., 2013 [64]	42 adults	4 g/d EPA + DHA	12 weeks	Reduction in serum TG concentrations (mean $\pm$ SEM) was $-26 \pm 4\%$ (-0.81 ± 10.12 mmol/L);	Total LDL-C concentration increased by $13 \pm 3\%$ ($+0.31 \pm 0.08$ mmol/L); Changes in LDL phenotype patterns A, B and A/B were negligible and not statistically significant.
Haghravan et al., 2016 [47]	44 overweight women aged between 20 to 45 years.	(1) received omega 3 supplements (600 mg EPA plus 300 mg DHA), aerobic exercise, and diet education. (2) received placebo capsules, aerobic exercise, and diet education.	8 weeks	–	Body weight, BMI, body fat percentage, abdominal circumference and abdominal skinfold thickness diminished in omega-3 groups. Omega 3 improved VO ₂ max outcome compared to that of the control group.

Table 7. Studies assessing vitamin D status in countries located in northern latitudes.

Study	Subject characteristics	Location	Methods	Vitamin D (nmol/l) mean of range	
				Winter	Summer
Rockell et al., 2006 [41]	2946 man and woman	New Zealand	RIA Diasorin.	38 44	79 67
Bolland et al., 2007 [42]	1606 healthy postmenopausal women and 378 older men	New Zealand	Serum 25(OH)D was measured by RIA in all the women and the first 252 men; a chemiluminescent was used in the last 126 men.	50–59 in men, 50–57 in woman.	90 in men and 70 in women.
Hypponen et al., 2007 [43]	7437 participants, 45 y old.	Great Britain	25(OH)D was measured by using automated application of an enzyme-linked immunosorbent assay.	41.1 in man, 41.2 in woman	61.9 in man, 58.6 in woman
Hill et al., 2008 [44]	1015 of 12 and 15 year-old boys and girls	Northern Ireland	Serum concentrations of 25-hydroxyvitamin D (25(OH)D) were analysed by enzyme-immunoassay. Overall mean 25(OH)D concentration throughout the year.	56.7	78.1
Kull et al., 2009 [45]	367 individuals (200 women and 167 men) mean age 48.9 ± 12.2 years	Estonia	25-(OH) vitamin D (25(OH)D) level and parathyroid hormone (PTH) were measured in summer and in winter.	43.7 ± 15	59.3 ± 18
Andersen et al., 2013 [46]	54 girls (11–13 years) and 52 women (70–75 years).	Denmark	The participants were examined three times (February–March 2002 (winter-1), August–September 2002 (summer) and February–March 2003 (winter-2).	23.4 (winter-1), 29.5 (winter-2) in girls and 47.2 (winter-1), and 50.5 (winter-2) in woman	60.3 in girls, 67.3 – in woman

risk factors in athletes [58, 59]. A study of 16 well-trained male cyclists indicated that fish oil supplementation may act within the healthy heart and skeletal muscle to reduce

both whole-body and myocardial O₂ demand during exercise, without a decrement in performance [60]. There is some evidence that omega-3 fatty acid can modestly

enhance lipolysis and β -oxidation during exercise and thereby improve fat loss [61]. There is also some evidence that omega-3 supplementation may help to improve various aspects of exercise performance. However, limitations in study design make it difficult to draw firm conclusions on these topics [61].

The limitation of our study is body composition analysis. We should also have used anthropometric circumference (waist and hip circumference measurement) for better demonstration of changed body fat. Future studies may include more anthropometric measurements, diet, physical activity status evaluations during the research.

Conclusion

The daily supplementation of 330 mg of omega-3 fatty acid for 16 weeks is lower than the 500 mg of omega-3 fatty acid dose recommended by the International Society for the Study of Fatty Acids and Lipids [42] and the European Food Safety Authority [43] to maintain good health. Our findings indicate that while there was a positive impact on total cholesterol level and glucose level, there was no effect on glucose, LDL, HDL, or TG levels. The daily dose of 330 mg can therefore be seen as insufficient.

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Conflict of Interests

The authors declare that they have no competing interests.

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