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Risk factors associated with plasma omega-3 fatty acid levels in patients with suspected coronary artery disease

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ABSTRACT

Introduction: We sought to determine the associations between plasma eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) levels and various cardiovascular risk factors and with the use of fish oil supplements (FOS).

Patients and methods: Patients with suspected coronary artery disease (CAD) undergoing cardiac catheterization (n=433) were studied. Serum fatty acid (FA) composition, the concentrations of lipids and biomarkers of oxidative stress, and dietary/lifestyle factors were measured.

Results: FOS use was associated with a higher plasma EPA+DHA levels (3.7 ± 1.5 vs. $2.6 \pm 1.1\%$, $p < 0.0001$). However, there was no relationship between FOS dose (mg/day) and EPA+DHA levels in 76 patients reporting FOS use ($r = -0.21$, $p = 0.07$). Lower levels were inversely associated with risk factor profiles including lower ApoB100/ApoA1 ratios ($p < 0.001$).

Discussion and conclusions: Higher EPA+DHA levels characterized patients with lower CAD risk. The lack of relations between FOS dose and plasma EPA+DHA levels likely reflects uncaptured variability in EPA+DHA content of supplements.

1. Introduction

Multiple studies have shown that marine-derived, polyunsaturated, long chain omega-3 fatty acids (FA) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) reduce the risk of adverse cardiovascular (CV) events such as sudden death, thrombosis, myocardial infarction, and arrhythmia in patients with cardiovascular disease (CVD) [1–3]. There is also evidence that EPA and DHA reduce the risk of neuro-psychiatric disease [4]. As therapeutic agents, encapsulated omega-3 products [including fish oil supplements (FOS) and pharmaceutical preparations] are well-known to lower plasma triglyceride, very low density lipoprotein (VLDL) and intermediate density lipoprotein (IDL) levels [5,6]. FOS have also been shown to increase concentrations of high density lipoprotein (HDL) particles [7], with a shift towards larger, more buoyant low density lipoprotein (LDL) [8], all of which are associated with a reduced risk for CV events [9,10].

Epidemiological studies have demonstrated a strong association between higher omega-3 FA intakes and/or blood levels and reduction in the risk of CVD [11]. However, results from several randomized trials and a recent meta-analysis by Rizos et al. [12], have failed to show a benefit of omega-3 supplementation on CV events (except for cardiac death). A potential explanation [13–15] for this observed discordance could be unmeasured confounding in observational cohort studies. The baseline demographics of individuals who consume more fish and/or have higher circulating omega-3 FA levels may be fundamentally different from non-consumers which might be a contributing factor in their lower risk of CV events [16]. We aimed to explore the differences between CV risk and lifestyle factors amongst patients with suspected coronary artery disease (CAD) who have higher plasma omega-3 FA levels compared to those with lower levels. We further explore if FOS intake is reflected in plasma levels of omega-3 FA.

2. Materials and methods

This is a retrospective analysis of data from an original study designed to examine the association between self-reported FOS

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use and several CV risk markers [17]. Consecutive patients (n=433) > 18 years old with suspected CAD undergoing elective cardiac catheterization on 325 mg daily aspirin therapy were enrolled in the Multi-Analyte, Thrombogenic, and Genetic Markers of Atherosclerosis (MAGMA) study (NCT01276678) between July 2010 and May 2014. Written informed consent was obtained, and the study was approved by Institutional Review Board. Patients were referred for elective cardiac catheterization for the following reasons: (1) a positive stress test with or without classic angina; and/or (2) a positive CT scan. Inclusion and exclusion criteria and collection of non-laboratory data are detailed elsewhere [17] as are the procedures for blood and urine collection and subsequent laboratory analysis.

2.1. Measurement of omega-3 fatty acids

EPA and DHA content in plasma is expressed as a percent of total FA (i.e., 24 FA; Supplemental Data: Table 1) and is determined as previously reported [18]. Briefly, samples were thawed, and an aliquot was combined (1:40 parts) with the methylating mixture (boron trifluoride in methanol [14%], toluene, and methanol [35/30/35 v/v]), shaken and then heated at 100 °C for 45 min. After cooling, 40 parts of both hexane and distilled water were added. After briefly vortexing, the samples were spun to separate layers, and an aliquot of the hexane layer that contained the fatty acid methyl esters was analyzed by gas chromatography. Additionally, in order to estimate what *erythrocyte membrane* EPA+DHA levels (the Omega-3 Index) would have been in these patients, we correlated plasma EPA+DHA levels with the Omega-3 Index in 50 randomly selected clinical samples. The Omega-3 Index method has been described elsewhere [18].

2.2. Statistical analysis

Categorical variables are expressed as number (percentage) and continuous variables as mean ± SD or median (interquartile range) as appropriate. Fischer's exact or Chi Square tests were used for comparisons of categorical variables. Student's *t* test was utilized for normally distributed continuous datasets, while Welch's *t*-test was used for continuous datasets that did not follow a normal distribution. Plasma levels of EPA+DHA (%) were divided into groups for analysis at approximately tertile cut-points. The lowest tertile was below 2.14% and the highest tertile began at 2.97%. Accordingly, convenient boundaries of < 2%, 2–3% and > 3% were used in this analysis. One-way ANOVA analysis was used to compare risk markers and lifestyle factors across EPA+DHA groups. Association between fish oil dose, plasma EPA+DHA levels, patient characteristics (age, sex, race, BMI, weight, smoking, diabetes, kidney and liver diseases, cancer, hypertension, thyroid disease, history of myocardial infarction, stroke and peripheral vascular disease) and plasma and erythrocyte membrane EPA+DHA levels were determined using regression analysis (version 13.1.2, Medcalc Statistical Software, Ostend, Belgium). A *p*-value of ≤ 0.01 was considered statistically significant to partially control for multiple testing.

3. Results

3.1. Baseline demographics

A total of 433 patients were included in this study. The population primarily consisted of Caucasian males with multiple CV risk factors. The significant differences in the distribution of comorbidities across groups are listed in Table 1.

Table 1
Baseline demographics.

		Approximate EPA+DHA tertiles			
	All (n=433)	**< 2% (n=118)**	**2%–3% (n=172)**	**> 3% (n=143)**	
	Mean (SD)	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	***p*-Value***
Demographics and Biometrics					
Age (yrs)	63.3 (10.3)	61.6 (11.7)	62.7 (10.0)	65.5 (9.2)	**0.006**
Gender (male %)	63.8	69.5	57.6	67.1	0.06
Weight (kg)	90 (20)	93 (22)	92 (20)	87 (18)	0.02
Body mass index (kg/m^2)	30.9 (6.6)	31.3 (7.5)	31.6 (6.7)	29.8 (5.5)	0.04
Waist Circumference (cm)	101 (17)	104 (22)	103 (16)	97 (14)	**0.01**
Dietary habits, medications and smoking					
Soda (8-oz cans/day)	1.1 (1.8)	1.6 (2.4)	1.0 (1.5)	0.9 (1.4)	**0.006**
Fast food (meals/week)	1.2 (1.7)	1.5 (2.1)	1.2 (1.7)	0.8 (1.2)	**0.009**
Low fat diet (%)	24.3	23.6	21.1	35.8	**0.01**
Active smoker (%)	14.0	22.7	11.4	9.6	**0.006**
Statin use (%)	66.1	55.9	66.9	74.1	**0.006**
Fish Oil supplement use (%)	22.9	5.3	15.6	47.5	< **0.001**
Medical History (%)					
Myocardial infarction	23.0	25.6	22.4	22.5	0.77
Peripheral Vascular disease	9.5	9.3	9.6	9.9	0.98
Hyperlipidemia	77.7	76.3	76.6	80.4	0.66
Hypertension	77.0	75.4	78.4	76.9	0.88
Stroke	7.6	6.8	7.1	9.1	0.72
Diabetes	60.8	38.1	29.8	34.2	0.31
Liver disease	26.4	6.6	1.8	3.0	0.32
Kidney disease	25.9	6.6	3.1	3.0	0.58

* $p \leq 0.01$ considered statistically significant.

3.2. Plasma EPA+DHA groups and lifestyle variables

The number of patients included in each approximate tertile (< 2%, 2–3% and > 3%) was 118, 172, and 143, respectively. The patients in the lowest group were younger and had a larger waist circumference than patients in the highest group. They also consumed more soda per day, fast food meals per week and were more likely to be current smokers compared to highest group. The patients in the highest group were more likely to eat a low-fat diet, to use statins and FOS compared to lowest group ($p \leq 0.01$ for all) (Table 1).

The 101 patients on FOS had higher plasma EPA+DHA than the 332 patients not on FOS (3.88 ± 1.53% vs. 2.49 ± 0.93% respectively, $p < 0.001$). Data on the reported dose (i.e., mg of oil, not EPA+DHA, taken per day) were available for 76 of the FOS patients. Among these patients, the median daily dose of fish oil was 2000 mg (range 1000–4800 mg). There was a borderline significant inverse correlation between the plasma EPA+DHA levels and daily reported dose of FOS (r = −0.21, 95% CI −0.41 to 0.02; $p = 0.07$, Fig. 1). The mean plasma EPA+DHA levels were 2.61 ± 1.19% in the non-statin group (n=147) and 2.92 ± 1.26% in the statin users (n=286, $p = 0.014$). This difference was observed in patients who were not taking FOS (n=332; 2.32 ± 0.90% vs 2.58 ± 0.93%, $p = 0.01$), and a similar trend was seen for the smaller group taking FOS (n=111; 3.52 ± 1.54% vs 4.07 ± 1.51, for statin non-users and users respectively, $p = 0.08$).

Logistical regression was performed to find independent predictors of elevated plasma EPA+DHA (i.e., levels > 3% vs ≤ 3%).