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Clinical Correlates of Red Blood Cell Omega-3 Fatty Acid Content in Male Veterans with Peripheral Arterial Disease

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Abstract

Objectives—Despite available medical therapies, patients with peripheral arterial disease (PAD) remain at high risk for cardiovascular events. *n*-3 polyunsaturated fatty acids (PUFA), derived from marine sources, have been shown to improve cardiovascular mortality. The omega-3 index (O3I), a proportion of the *n*-3 PUFA eicosapentaenoic acid and docosahexaenoic acid in the red blood cell membrane, correlates with cardiovascular risk. Previous investigations have found that *n*-3 PUFA supplementation, fish consumption, older age and smoking history affect the O3I in different patient populations, though similar correlations have never been explored in PAD. We hypothesized that in our PAD cohort, blood content of omega-3 fatty acids would directly and positively correlate with a history of fish oil supplementation and older age, and inversely correlate with a smoking history and obesity

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CONFLICTS OF INTEREST/DISCLOSURES

WSH is the President of OmegaQuant Analytics, LLC and a Senior Research Scientist and Health Diagnostic Library, Inc., both of which offer the blood omega-3 testing. The remaining authors do not have anything to disclose.

Methods—This cross-sectional study included 111 patients, who had an ankle-brachial index of <0.9 associated with claudication symptoms. We used linear regression to determine the association between clinical factors and the O3I.

Results—The mean age of the cohort was 69 ± 8 years, 37% had diabetes mellitus (hemoglobin A1c: 7 ± 1%), and 94% reported current or a history of smoking. The mean O3I was 5 ± 2%. In multivariate linear regression analysis, O3I was associated with older age, increasing BMI, and a history of smoking and fish oil intake.

Conclusions—This is the first report of the relation between blood content of omega-3 fatty acids and clinical factors in a PAD population. In patients with PAD, older age, elevated BMI, and prior fish oil supplementation predicted a higher O3I. A history of smoking correlated with a lower O3I. These results demonstrate that the O3I is a reliable measure of dietary n-3 PUFAs intake and that clinical factors related to the O3I in PAD are similar to those observed in other populations.

INTRODUCTION

In a primary care setting, nearly one-third of patients aged 70 and older will suffer from peripheral artery disease (PAD),[1] which significantly impacts their quality of life and longevity. Despite the available medical therapies, patients with PAD continue to have a higher risk of cardiovascular events compared to patients with coronary artery disease (CAD).[2] In addition to traditional risk factors such as diabetes mellitus, smoking, hyperlipidemia and hypertension, a growing body of evidence indicates that inadequate nutrient intake and excessive caloric intake contribute to the development of PAD among other cardiovascular diseases.[3–6] Lower blood levels of omega-3 polyunsaturated fatty acids (*n*-3 PUFA), an essential nutrient, may be involved in the development of PAD.[7]

The omega-3 index (O3I)—which is a measure of red blood cell (RBC) content of the two major long-chain *n*-3 PUFA, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)[8] expressed as a percentage of total RBC fatty acids—has become a validated marker of tissue *n*-3 PUFA content and an independent, graded risk factor for death from CAD.[9] We previously demonstrated that the O3I was inversely associated with inflammatory status in patients with PAD.[10] Our data also demonstrate that supplementation with *n*-3 PUFA leads to decreased adhesion of monocytes to the endothelial monolayer *in vitro*.[11]

Prior studies that evaluated clinical determinants of the O3I in patients at risk for CAD found that a history of *n*-3 PUFA supplementation had the strongest positive predictive value, followed by non-fried fish consumption and older age; smoking history had a strong negative predictive value.[12,13] This study aimed to determine the clinical, anthropometric, and lifestyle factors associated with the O3I in patients with PAD because this patient population is at greater cardiovascular risk than patients with CAD or the general population. A better understanding of these associations could help guide future therapeutic interventions, such as personalized dosing recommendations, in an effort to improve PAD patients' vascular function and prognosis. Based on the associations observed by previous investigators, we hypothesized that in our PAD cohort, blood content of omega-3 fatty acids

would directly and positively correlate with a history of fish oil supplementation and older age, and inversely correlate with a smoking history and obesity.

METHODS

Study Population and protocol

This study utilized baseline data from a prospective trial investigating the effects of *n*-3 PUFA supplementation on inflammatory markers and vascular function in PAD patients.[10] The investigator-initiated protocol was approved by the Committee on Human Research at the University of California, San Francisco (UCSF) and all patients provided informed consent. Patients referred to the outpatient vascular surgery clinic of the San Francisco Veterans Affairs Medical Center (SF VAMC) for evaluation of PAD were screened for the study.

PAD diagnosis was based on current guidelines of an index ankle-brachial index (ABI) <0.9 on the affected limb(s) at presentation with the presence of claudication.[14] We excluded patients with incompressible arteries or an ABI >1.4. None of the participants had pain at rest or tissue loss. Claudication was diagnosed based on fatigue, discomfort, or pain that occurs in specific limb muscle groups during effort due to exercise-induced ischemia.[15] The study exclusion criteria included: significant renal (Creatinine level ≤ 2 mg/dL), hepatic (active or acute hepatic disease, though non-active chronic hepatitis C was not considered an exclusion criteria), or inflammatory disease (eg. Crohn's disease, Ulcerative colitis, Primary biliary cirrhosis, Sclerosing cholangitis, etc.), concurrent severe infections, acute illness or other major surgery within 30 days of evaluation or ingestion of immunosuppressive medications. Those meeting inclusion criteria were invited to enter the research study, at which time informed consent was obtained by study staff in accordance with requirements of the Committee for Human Research. Data on demographics (age, race, and gender), anthropometrics (hip and waist circumference, body mass index), lifestyle (prior supplement use and cigarette smoking), cardiovascular (CVD) disease history [e.g., coronary artery disease (CAD) and previous CV procedures), CVD risk factors (hypertension, diabetes, hypercholesterolemia, and cigarette smoking), medications, and pertinent vascular examination findings were recorded. Circulating biomarkers were also measured, including the O3I, inflammatory markers (CRP, IL-6, TNF-α), and lipid panel [LDL-Cholesterol (C), triglycerides, HDL-C], blood pressure, and bilateral ABI. A validated food frequency questionnaire was also administered to estimate dietary intake.[16]

Measurements

RBC n-Fatty Acids—To measure the O3I, whole venous blood was collected in a fasting state in an EDTA tube, and centrifuged at 2800 rpm for 10 minutes at 4C° within 30 minutes of collection. After removal of the plasma and buffy coat, packed RBCs were stored at −80C until assayed as previously described.[17] Among the fatty acids quantified were *n*-3 and *n*-6 FAs, arachidonic acid (AA), EPA, DHA, omega-3 index, saturated FA, *trans*-FA, and monounsaturated FA. FA methyl esters are generated from RBCs by acid transesterification with boron trifluoride and analyzed by capillary gas chromatography using a GC2010 Gas Chromatograph (Shimadzu Corporation, Columbia, MD) equipped with a SP2560, 100-m

column (Supelco, Bellefonte, PA). FA were identified by comparison with a standard mixture of FA characteristic of erythrocytes and reported as a percentage of total identified FA after response factor correction. C18-*trans* FA were defined as the sum of the *trans*-isomers of oleic acid (C18:1) and linoleic acid (C18:2n-6). The typical coefficient of variation for the Omega-3 Index (EPA+DHA) using this procedure is 3% and 7% for *trans*-FA.

Inflammatory Markers—Inflammatory markers studied included CRP, IL-6, and TNF-α. These biomarkers were chosen based on their association with disease severity in PAD.[18–21] Whole venous blood was collected in a fasting state in an SSTP tube, allowed to clot for a minimum of 30 minutes at room temperature, and centrifuged at 2800 rpm for 10 minutes at 4C°. Serum was stored at −80C° until assayed for IL-6, and TNF-α per standard kit protocol (R&D Systems Inc., Minneapolis, MN). The typical coefficients of variation for IL-6, and TNF-α are 7.4% and 5.4%, respectively. The lower limits of detection are 0.04pg/ml and 0.11pg/ml, respectively. Plasma obtained from the EDTA tubes described above was assayed for CRP the same day as collection by the SF VAMC lab per standard methodology (Beckman Coulter Analyzer, Miami, FL). The coefficient of variation for CRP using this procedure is 5.1%.

Ankle-Brachial Index—The ABI was measured using current guidelines and standards.[14] ABI measurements were collected by trained vascular clinical staff of the Vascular Integrated Physiology and Experimental Therapeutics (VIPERx Lab – please see www.viperxlab.org for more details), which is run by accredited and licensed vascular surgeons. Systolic blood pressures of the brachial, posterior tibial and dorsalis pedis arteries were measured bilaterally. For each lower extremity, the highest systolic pressure of the two pedal pulses was divided by the highest systolic pressure of the two brachial arteries.

Renal, Lipid and Metabolic Measurements—EDTA blood samples were collected in a fasting state for measurement of creatinine (Cr), estimated glomerular filtration rate (eGFR), albumin, total cholesterol, triglycerides, low-density lipoprotein, high-density lipoprotein, and hemoglobin A1C (Hgb A1C) if patients were diabetic. Plasma was assayed for these analytes on the same day as collection by the SF VAMC lab per standard methodology (Beckman Coulter Analyzer). Serum was isolated at the same time points as described above and assayed for homocysteine on the same day as collection by the SF VAMC lab per standard methodology (Abbott Diagnostics Architect i1000 Analyzer, Lake Forest, IL).]

Statistical Analysis

For descriptive purposes, we categorized participants by tertiles based on the O3I. Differences in baseline characteristics between these groups were compared using analysis of variance (ANOVA) for continuous variables, the χ^2-test for categorical dichotomous variables, and the Kruskal-Wallis test for categorical variables with multiple designations (e.g., Rutherford classification). Since inflammatory markers and homocysteine had a skewed distribution, they were log-transformed for subsequent statistical analyses. For regression modeling, the O3I was used as a continuous variable. We used multivariable

linear regression models to estimate the relationship between the O3I and various clinical, lifestyle and anthropometric factors. We employed a stepwise process to construct the multivariable model, incorporating the independent predictor variables from univariate analysis as well as HDL-C, LDL-C, and triglyceride levels, factors found to significantly correlate with the O3I in a previous study by Block et al.[12] Covariates associated with the O3I at P<.05 were retained. Statistical analyses were performed using Stata/SE 12 (StataCorp, College Station, TX).

RESULTS

A total of 111 patients with PAD were recruited for the study, and all reported lifestyle-limiting lower extremity claudication. The mean age of the cohort was 69 ± 8 years, 40% had CAD, 37% had diabetes mellitus (hemoglobin A1c: 7 ± 1%), and 94% reported current or a history of smoking. The mean O3I was 5.2 ± 1.8%. The demographics, medications, and lifestyle characteristics of the population and laboratory studies are summarized in Table 1 by O3I tertiles. The patients with a higher O3I were more likely to be older and lack a smoking history. There was a trend for subjects with increasing O3I levels to have a higher BMI. A greater proportion of patients with a higher O3I also reported supplementation with *n*-3 PUFA.

The constituent fatty acids that make up the red blood cell membrane also differed across O3I tertiles (Table 2). Patients with a higher O3I were more likely to have higher levels of *n*-3 PUFA, and in particular EPA and DHA. A lower O3I was associated with a greater proportion of *n*-6 PUFA. Levels of saturated, trans, and monounsaturated fatty acids were relatively equal across the three tertiles.

The results from univariate and subsequent multivariable regression analyses are detailed in Table 3. In univariate analyses, older age (P-value: .003), increasing BMI (P-value: .05), a diagnosis of hypertension (P-value: .03), and a history of *n*-3 PUFA supplementation (P-value: <.001) were found to positively correlate with the O3I. A history of smoking (P-value: .003) correlated with a lower O3I. In a multivariable model, an independent association remained with age by each successive decade (P-value: .001), BMI increasing by intervals of 5 (P-value: .001), a history of smoking or current smoking (P-value: .03), and supplementation with *n*-3 PUFA (P-value: .001). To further explore the association with higher BMI and the O3I, we re-categorized BMI into two groups: BMI > 30 (N= 37 out of 111) and BMI < 30 (N=74). When running a univariate analysis to only include the 37 patients with a BMI > 30, there was no longer a significant correlation with the O3I. We further re-categorized the data into BMI > 35 (N= 12) and BMI < 35 (N=99) and similarly, observed no significant association with the O3I.

DISCUSSION

In a prospective cohort study of patients presenting for evaluation of PAD in an outpatient vascular surgery clinic, we found that older age, increasing BMI, and prior fish oil supplementation predicted a higher O3I. A history of smoking correlated with a lower O3I.

These findings confirm associations observed in non-PAD populations. Our observations suggest that targeting dietary fish consumption and fish oil supplementation as well as tobacco use with therapeutic interventions has potential to reduce cardiovascular risk among PAD patients.

A decade ago, Harris and von Schacky proposed the O3I as a biomarker of *n*-3 PUFA status, on the basis that the proportional EPA + DHA content in red blood cell membranes provides a good estimation of *n*-3 PUFA intake.[22] Other studies demonstrated the utility of measuring blood levels of long-chain PUFA for the purpose of cardiovascular disease risk stratification.[23–25] A lower O3I has been shown to be predictive of acute coronary syndrome (ACS),[12,25] primary cardiac arrest,[23] and sudden cardiac death.[24] Based on these associations, Harris et al. proposed graded O3I risk zones for CAD, defined as high risk, intermediate, low, and corresponding to <4%, 4%–8%, >8%, respectively. Compared to patients with CAD, those with PAD have a more pronounced inflammatory phenotype and are at increased risk for cardiovascular events. PAD patients also have significantly elevated risks of limb amputation and premature mortality compared to the general population.[1,26] To our knowledge, a characterization of O3I levels among patients with PAD has not yet been described. Therefore, we aimed to estimate the relative levels of *n*-3 PUFA with the O3I in a PAD cohort and determine associated clinical risk factors.

Prior reports have attempted to define determinants of the O3I in at-risk populations. The first was a cross-sectional analysis of outpatients.[12] The most significant predictors of a favorable O3I were EPA + DHA supplement use and frequency of non-fried fish consumption. Older age and a history of dyslipidemia were also associated with a higher O3I, while factors that negatively associated with the O3I were smoking history and triglyceride level. Another cross-sectional study by Sala-Vila et al. included subjects from Spain with several risk factors but without diagnosed cardiovascular disease or PAD.[13] The authors noted that rates of cardiac death among the Mediterranean population in general are relatively low despite a high prevalence of risk factors. They hypothesized that the regional diet may be protective due to increased intake of seafood, which is a rich source of EPA and DHA. Consistent with this hypothesis, their study cohort had an average O3I of 7.1%, which is higher than the reported average of approximately 5% in other Western populations.[12] After adjusting for demographic and lifestyle factors and energy expenditure, they also found that EPA + DHA intake was the principal O3I determinant.

The strongest predictor of a higher O3I in the current study was a history of supplementation with *n*-3 PUFA. This finding is consistent with the results of Block et al. and Sala-Vila et al. and provides further justification that the O3I is a good reflection of dietary intake of *n*-3 PUFA. Furthermore, in a recent dose-response randomized controlled trial that investigated supplementation with *n*-3 PUFA in young and healthy subjects, Flock et al. found that the O3I increased in a dose-dependent manner.[27] Baseline O3I, older age, gender, and physical activity level were also associated with a higher O3I. Interestingly, when they adjusted per unit body weight (grams/kilogram), the authors found that the dose of EPA+ DHA was still a powerful univariate predictor of O3I. They also observed that subjects who weighed less and were on higher doses experienced the greatest rise in their O3I. This finding brings up an important consideration for achieving an optimal O3I: that dosing recommendations for

n-3 PUFA should eventually be tailored to an individual's demographics and clinical history rather than being standardized for the general population, as they are now.

Another strong predictor of O3I in this study was having a smoking history, and it predicted a lower O3I. Although smoking was highly prevalent in our cohort (94% reported a history of smoking with average pack-years 47 ± 35), significant differences were noted across the O3I tertiles: 100% of individuals with the lowest O3I were smokers compared to 84% with the highest O3I. A plausible reason for this correlation is that cigarette smoking likely induces a pro-oxidative state,[28] especially if it is a chronic practice, and this may lead to destruction of membrane PUFA.[29] Supplementing with *n*-3 PUFA may be protective because these fatty acids readily incorporate into cellular phospholipids, which leads to a relative reduction of *n*-6 PUFA; a resulting increased *n*-3 to *n*-6 PUFA ratio has been shown to correlate with improved endothelial function.[30] Also, *n*-3 PUFA supplementation could lead to recovery of endothelial synthesis of nitric oxide and prostaglandin I2, as well as vascular smooth muscle cell sensitivity to nitric oxide. Siasos and colleagues demonstrated that addition of *n*-3 PUFA may attenuate the detrimental oxidative effects that result from smoking. In their study of young and healthy smokers, the authors found that endothelial function significantly improved after oral treatment with 2 grams/day of *n*-3 PUFA for several months.[31] Their results indicate that supplementation could, in theory, lead to benefits in patients with PAD and should be examined more prospectively. In another study that prospectively evaluated older male smokers, current smoking status was associated with development of claudication, whereas intake of *n*-6 PUFA and certain anti-oxidants conferred a reduced risk of claudication onset.[6] Whether smoking history is predictive of the O3I is not yet conclusive because other studies have found varying correlations.[32,33] Therefore, this relation needs to be explored further.

In our cohort of primarily Caucasian and male veterans with PAD, older age predicted a higher O3I in univariate analysis and showed an even stronger correlation after adjusting for *n*-3 PUFA supplementation and other potential confounders. This association has been reported previously and in several different populations.[12,17,34,35] Because older age is more associated with chronic conditions requiring medication use, this may imply an increased likelihood of taking supplements. Previous authors have reported a biochemical basis[35] for the higher O3I observed in older age, noting that potential mechanisms may include preferential incorporation of *n*-3 PUFA relative to other fatty acids[36] and a declining turnover rate of membrane *n*-3 PUFA.[12] Further research is required to better understand the biochemical and physiologic mechanisms for the age-related differences the O3I.

Despite attempts at treatment with available medical therapies, patients with PAD continue to have a higher risk of cardiovascular events compared to patients with CAD alone.[2,37] Our findings are significant for showing factors that have been reported to correlate with the O3I in non-PAD populations also apply to those with PAD. Consequently, counseling PAD patients about changes in diet to include more naturally occurring *n*-3 PUFA or supplementing with greater doses of *n*-3 PUFA may increase their O3I, and could potentially improve their symptoms and prognosis. Additional prospective trials are warranted to determine if dietary supplementation can improve clinical outcomes in this population.

Limitations

This cross-sectional study was observational in nature and included a relatively small sample size. We also lacked data on the exact amounts of prior EPA + DHA supplementation, as well as fish consumption. The patient population studied was not representative of the broader PAD population as it included predominantly male Caucasian veterans from SF VAMC. Gender could not be controlled for due to the two females included in the analysis. In addition, our study did not include patients with asymptomatic or premature PAD, or any individuals with more advanced stages of the disease, eg. those with renal and/or hepatic failure.

CONCLUSIONS

In a cohort of patients with PAD, the O3I was positively associated with a history of supplementation with *n*-3 PUFA, older age, and higher BMI. A negative correlation existed with smoking history. Additional large, prospective studies are needed to determine if manipulation of the O3I via dietary changes or fish oil supplementation could improve symptoms, vascular function or cardiovascular risk in this population.

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Table I

Baseline characteristics of the population categorized by Omega-3 Index tertiles

Descriptive Characteristics	**All patients (N= 111)**	**O3I tertile I (2.79 – 4.10) (N= 37)**	**O3I tertile II (4.11 – 5.04) (N= 37)**	**O3I tertile III (5.06 – 12.33) (N= 37)**	**P-value[a]**
Age, years	69 ± 8	66 ± 7	69 ± 8	71 ± 7	.02
Male sex, %	108 (97)	36 (97)	36 (97)	36 (97)	1.0
Caucasian race, %	84 (76)	29 (78)	27 (73)	28 (76)	.3
BMI, kg/m^2	28 ± 5	27 ± 5	28 ± 4	29 ± 6	.08
Waist-to-hip ratio, %	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	.3
Rutherford category, %					.4
Mild claudication	33 (30)	12 (32)	7 (19)	14 (38)	
Moderate claudication	32 (29)	12 (32)	10 (27)	10 (27)	
Severe claudication	44 (40)	12 (32)	20 (54)	12 (32)	
Blood pressure, mm Hg					
Systolic	137 ± 18	138 ± 18	134 ± 16	139 ± 20	.5
Diastolic	76 ± 10	76 ± 11	75 ± 9	76 ± 8	.9
Index ABI	0.7 ± 0.1	0.7 ± 0.2	0.7 ± 0.1	0.7 ± 0.1	.6
Comorbidities					
Hypertension	102 (92)	32 (86)	33 (89)	37 (100)	.08
Hyperlipidemia	96 (86)	30 (81)	32 (86)	34 (92)	.4
History of CAD	44 (40)	13 (35)	14 (38)	17 (46)	.6
Diabetes mellitus	41 (37)	11 (30)	13 (35)	17 (46)	.3
Medications/Supplements					
Aspirin	79 (71)	27 (73)	23 (62)	29 (78)	.3
ACE-inhibitor	51 (46)	14 (38)	19 (51)	18 (49)	.5
β-blocker	62 (56)	22 (59)	20 (54)	20 (54)	.9
Statin	92 (83)	30 (81)	29 (78)	33 (89)	.4
Insulin-use, if diabetic	15 (14)	3 (8)	6 (16)	6 (16)	.7
Hx of fish oil intake, %	38 (35)	10 (28)	8 (22)	20 (56)	.005
PAD risk factors					
Hx of smoking, %	104 (94)	37 (100)	36 (97)	31 (84)	.009

Descriptive Characteristics	All patients (N= 111)	O3I tertile I (2.79 – 4.10) (N= 37)	O3I tertile II (4.11 – 5.04) (N= 37)	O3I tertile III (5.06 – 12.33) (N= 37)	P-value[a]
Pack-years, if applicable	47 ± 35	52 ± 38	51 ± 38	37 ± 25	.2
Cholesterol, mg/dL					
Total	160 ± 41	168 ± 41	161 ± 40	152 ± 42	.2
LDL	87 ± 36	92 ± 35	87 ± 34	81 ± 39	.4
HDL	45 ± 14	43 ± 12	45 ± 16	46 ± 13	.6
Triglycerides, mg/dL	149 ± 91	173 ± 108	147 ± 88	126 ± 68	.1
Laboratory studies					
Ω-3 Index, %	5.0 ± 1.8	3.6 ± 0.3	4.6 ± 0.3	6.8 ± 1.9	<.001
logCRP, mg/dL	1.0 ± 1.0	1 ± 1	1 ± 1	0.7 ± 1	.2
logHomocysteine, μmol/L	2.6 ± 0.3	3 ± 0.4	3 ± 0.3	3 ± 0.2	.008
eGFR, mL/min/1.73 m^2	73 ± 23	80 ± 24	67 ± 22	75 ± 21	.07
Creatinine, mg/dL	1.2 ± 0.7	1 ± 0.4	1 ± 0.4	1 ± 1	.5
Albumin, g/dL	4.0 ± 0.3	4 ± 0.3	4 ± 0.4	4 ± 0.3	.2

[a] p-value calculated from ANOVA for continuous variables or either the Kruskal-Wallis tests or χ^2-test for categorical variables

Mean ± SD presented for continuous variables; Number of patients with relative % of cohort presented for categorical variables.

Table II

Constituent fatty acids of the red blood cell membrane, by O3I tertiles

Fatty Acid, % cell membrane	**All patients (N= 111)**	**O3I tertile I (N= 37)**	**O3I tertile II (N= 37)**	**O3I tertile III (N= 37)**	**P-value[a]**
Ω-3 Fatty acids, total	8 ± 2	6 ± 0.5	7 ± 1	10 ± 2	<.001
Alpha-linolenic C18:2n3	0.1 ± 0.07	0.1 ± 0.04	0.1 ± 0.05	0.1 ± 0.1	.3
Eicosapentaenoic C20:5n3	0.6 ± 0.5	0.4 ± 0.1	0.5 ± 0.2	1 ± 1	<.001
Docosapentaenoic-n3 C22:5n3	3 ± 1	3 ± 0.4	3 ± 0.4	3 ± 0.7	<.001
Docosahexaenoic C22:6n3	4 ± 1	3 ± 0.4	4 ± 0.3	6 ± 1	<.001
Ω-6 Fatty acids, total	35 ± 3	37 ± 2	36 ± 1	33 ± 3	<.001
Linoleic C18:2n6	11 ± 2	11 ± 2	11 ± 1	10 ± 2	.07
Gamma-linolenic C18:3n6	0.1 ± 0.03	0.1 ± 0.03	0.1 ± 0.04	0.1 ± 0.02	.01
Eicosadienoic C20:3n6	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	.4
Dihomo-g-linolenic C20:3n6	2 ± 0.4	2 ± 0.4	2 ± 0.4	2 ± 0.5	.9
Arachidonic C20:4n6	17 ± 2	18 ± 1	18 ± 1	16 ± 2	.01
Docosatetraenoic C22:4n6	5 ± 1	5 ± 1	5 ± 1	4 ± 1	<.001
Docosapentaenoic C22:5n6	1 ± 0.3	1 ± 0.2	1 ± 0.2	1 ± 0.3	.003
Saturated fat, total	41 ± 1	41 ± 1	41 ± 1	41 ± 1	1
Mysteric C14:0	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	.8
Palmitic C16:0	22 ± 1	22 ± 1	22 ± 1	22 ± 1	.9
Stearic C18:0	18 ± 1	18 ± 1	18 ± 1	18 ± 1	.7
Arachidic C20:0	0.1 ± 0.03	0.1 ± 0.04	0.1 ± 0.03	0.1 ± 0.03	.2
Behenic C22:0	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	.4
Lignoceric C24:0	0.3 ± 0.2	0.3 ± 0.2	0.3 ± 0.1	0.4 ± 0.2	.1
Trans fat, total	1 ± 0.4	1 ± 1	1 ± 0.3	1 ± 0.3	.01
Palmitelaidic C16:1n7t	0.1 ± 0.04	0.1 ± 0.04	0.1 ± 0.04	0.1 ± 0.04	.8
Elaidic C18:1t	1 ± 0.4	1 ± 0.5	1 ± 0.3	1 ± 0.2	.01
Linoelaidic C18:2n6t	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	.2
Monounsaturated fat, total	15 ± 1	15 ± 1	14 ± 1	15 ± 1	.04
Palmitoleic C16:1n7	0.3 ± 0.2	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	.2

Fatty Acid, % cell membrane	All patients (N= 111)	O3I tertile I (N= 37)	O3I tertile II (N= 37)	O3I tertile III (N= 37)	P-value[a]
Oleic C18:1n9	14 ± 1	14 ± 1	13 ± 1	14 ± 1	.07
Eicosenoic C20:1n9	0.2 ± 0.05	0.2 ± 0.05	0.2 ± 0.04	0.2 ± 0.06	.03
Nervonic C24:1n9	0.4 ± 0.2	0.4 ± 0.2	0.3 ± 0.1	0.4 ± 0.2	.04

[a] p-value calculated from ANOVA for continuous variables; Mean ± SD presented

Table III

Predictors of the Omega-3 Index in univariate and multivariate linear regression analysis (included if P ≤ .05)

Univariate Analysis; N=111					**Multivariate Analysis; N=84**			
Independent variable –	**Coefficient**	**95% Confidence-interval**		**P-value**	**Coefficient**	**95% Confidence-interval**		**P-value**
		Lower limit	**Upper limit**			**Lower limit**	**Upper limit**	
Age, by decade	0.6	0.2	1.0	.003	0.8	0.3	1.2	.001
History of smoking	−2.0	−3.3	−0.7	.003	−1.5	−2.7	−0.2	.03
BMI, by increments of 5	0.3	0.01	0.6	.05	0.5	0.2	0.8	.001
Hypertension	1.3	0.1	2.5	.03	NS	NS	NS	NS
History of *n*-3 supplem.	3.2	1.5	4.8	<.001	2.6	1.1	4.1	.001