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Peripheral Artery Disease is Associated with a Deficiency of Erythrocyte Membrane N-3 Polyunsaturated Fatty Acids

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Abstract

Population-based data suggest that individuals who consume large dietary amounts of n-3 polyunsaturated fatty acids (PUFA) have lower odds of peripheral artery disease (PAD); however, clinical studies examining n-3 PUFA levels in patients with PAD are sparse. The objective of this study is to compare erythrocyte membrane fatty acid (FA) content between patients with PAD and controls. We conducted a cross-sectional study of 179 vascular surgery outpatients (controls, 34; PAD, 145). A blood sample was drawn and the erythrocyte FA content was assayed using capillary gas chromatography. We calculated the ratio of the n-3 PUFA eicosapentaenoic acid (EPA) to the n-6 PUFA arachidonic acid (ARA) as well as the omega-3 index (O3I), a measure of erythrocyte content of the n-3 PUFA, EPA and docosahexaenoic acid (DHA), expressed as a percentage of total erythrocyte FA. Compared with controls, patients with PAD smoked more and were more likely to have hypertension and hyperlipidemia (p<.05). Patients with PAD had a lower mean O3I (5.0±1.7% vs. 6.0±1.6%, p<.001) and EPA:ARA ratio (0.04±0.02 vs. 0.05±0.05, p<.001), but a

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greater mean total saturated fats (39.5±2.5% vs. 38.5±2.6%, p=.01). After adjusting for several patient characteristics, comorbidities, and medications, an absolute decrease of 1% in the O3I was associated with 39% greater odds of PAD (OR 1.39, 95%CI 1.03–1.86 p=.03). PAD was associated with a deficiency of erythrocyte n-3 PUFA, a lower EPA:ARA ratio, and a greater mean total saturated fats. These alterations in FA content may be involved in the pathogenesis or development of poor outcomes in PAD.

Keywords

Lipid analysis; n-3 fatty acids; atherosclerosis

INTRODUCTION

Peripheral artery disease (PAD) is a global health problem that affects more than two hundred million people worldwide with evidence of increasing incidence (Fowkes et al., 2013). PAD is a disease of systemic atherosclerosis and has been associated with an increased risk of adverse cardiovascular events and mortality (Grenon et al., 2013) as well as high morbidity due to claudication symptoms, impaired quality of life, and risk of amputation (Breek et al., 2005, Regensteiner et al., 2008). Additionally, PAD has been associated with elevated levels of inflammation, and markers of systemic inflammation have been reported to predict mortality (Vidula et al., 2008) and poor surgical outcomes (Owens et al., 2007) among this population.

A higher consumption of n-3 polyunsaturated fatty acids (PUFA) has been reported to be associated with a lower prevalence of subclinical atherosclerosis (He et al., 2008), reduced inflammation (He et al., 2009), and lower cardiovascular and all-cause mortality (Marik et al., 2009). N-3 PUFA mediate their physiologic effects by being incorporated into cellular membranes, where they are capable of affecting the activity of several membrane proteins and characteristics (Harris, 2007, Harris, 2009). Once in the cellular membrane, n-3 PUFA can be released by intracellular phospholipases and then converted into a wide variety of bioactive lipid mediators, including the specialized pro-resolving lipid mediators (SPM) (Harris, 2007, Harris, 2009), which have been reported to modulate inflammation, vascular injury, and atherogenesis, all of which are key features of PAD (Serhan, 2014, Spite, 2013, Wu et al., 2017).

The omega-3 index is a measurement of erythrocyte membrane content of the predominant n-3 PUFA, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (von Schacky, 2011). It is also a simple and accurate measurement of long-term n-3 PUFA intake and correlates strongly with plasma and tissue n-3 PUFA content (Harris, 2008). A lower omega-3 index has been associated with increased systemic inflammation (Farzaneh-Far et al., 2009) and cardiovascular and all-cause mortality (Harris et al., 2018).

Population-based data suggest that individuals who report consuming large amounts of n-3 PUFA in their diets have lower odds of PAD (Lane et al., 2008); however, clinical assessments of n-3 PUFA erythrocyte membrane content and the omega-3 index in patients with PAD are limited. The objective of this study was to compare the composition of

membrane erythrocyte fatty acids between patients with and without PAD. We hypothesize that patients with PAD have a lower erythrocyte n-3 PUFA content, which may predispose these patients to atherogenesis, increased inflammation, and poor cardiovascular outcomes.

METHODS

Study Subjects

A cross-sectional sample of patients was recruited from the vascular surgery outpatient clinic at the San Francisco Veterans Affairs Medical Center (SFVAMC) between April 2011 and September 2016. PAD was defined as the presence of claudication symptoms and an abnormal ankle-brachial index (ABI; <0.9) or history of peripheral revascularization for claudication symptoms, regardless of ABI. Patients were defined as controls if they had a normal ABI (≥0.9) and no history of PAD or other known clinical atherosclerotic disease. Patients were excluded from participating if they were under 35 years old or had severe hepatic (Child-Pugh ≥ B), renal (creatinine ≥ 2 mg/dL), or non-vascular inflammatory disease. Patients were also excluded from participating if they had a severe acute illness within 30 days or were taking immunosuppressive medications or steroids. The Institutional Review Board (The Committee on Human Research) at the University of California, San Francisco and the SFVAMC Research and Development Office approved this study. Informed written consent was provided by all study participants.

Measurements

Demographics, Medical Comorbidities, and Anthropometrics—All subjects attended a study visit at the SFVAMC vascular surgery outpatient clinic and underwent a comprehensive vascular assessment. At this visit an intake questionnaire was administered that collected demographic variables including age, sex, and race. A comprehensive medical history and electronic medical record chart review was completed in order to determine the presence of several medical comorbidities including hyperlipidemia, hypertension, diabetes mellitus, and coronary artery disease (CAD). Medical comorbidities were recorded as being present if the patient had a documented history of the comorbidity in their electronic medical record that was then confirmed by the patient during the study visit. Information on smoking history and current medication use (aspirin, beta-blockers, statins, or ACE-inhibitors) was provided by the patient. Patients were directed to rest for 10 minutes before their brachial artery blood pressure was measured twice using a sphygmomanometer; the highest pressure was recorded. An ABI was measured in each lower extremity using previously established techniques (Grenon et al., 2009).

Laboratory Tests—At the same visit, a blood sample was obtained from each subject to measure lipids (triacylglycerols, high-density lipoprotein [HDL], low-density lipoprotein [LDL]), hemoglobin A1c (HbA1c), estimated glomerular filtration rate (eGFR), and high-sensitivity C-reactive protein (hsCRP). Each of these measurements were assayed for per standard methodology (Beckman Coulter Analyzer) on the same day as collection.

Omega-3 Index—The omega-3 index represents the erythrocyte content of the two major long-chain n-3 fatty acids, EPA and DHA, as a percent of total erythrocyte fatty acids

(Harris, 2008). Venous blood samples were collected at the study visit and stored at −80°C until assayed at a later date. Erythrocytes were then isolated from the venous blood sample and assayed for arachidonic acid (ARA), EPA, DHA, and other fatty acids as previously described (Harris et al., 2012). Fatty acids were generated by treatment with boron trifluoride-methanol and analyzed by capillary gas chromatography (GC2010 [Shimadzu Corp, Columbia, Maryland] equipped with a 100-m SP2560 column [Supelco; Bellefonte, Pennsylvania]). Fatty acids were then identified by comparison with a known standard (GLC-727; Nuchek Prep, Elysian, Minnesota).

Statistical analysis

All statistical analyses were performed using STATA version 15.0 (StataCorp, College Station, Texas). Continuous variables were inspected for normality prior to analysis. Summary statistics were reported using the mean and standard deviation for normally distributed continuous variables. Non-normally distributed continuous variables were summarized with their median and interquartile range. Categorical variables were described by frequency and percentage. Differences in baseline characteristics were compared between the PAD and control groups using a Student's *t*-test for normally distributed continuous variables, a Wilcoxon rank-sum test for those not normally distributed, and a Fisher's exact test for categorical variables.

An unadjusted univariate logistic regression model was used to calculate the odds ratio for PAD, using the omega-3 index as the primary predictor. Then, in order to adjust for patient characteristics, comorbidities, and medications that may be associated with increased odds of PAD, a multivariable model was built. Bivariate screening was used to select covariates for the multivariable model. In the bivariate analyses, the omega-3 index was used as the primary predictor of PAD, and all of the patient characteristics reported in Table 1 were examined individually as potential covariates. Any covariate associated at the level of $p \leq .20$ was included in the multivariable model. If correlated variables met criteria for inclusion (eg, HDL or LDL and hyperlipidemia), the underlying disorder (eg, hyperlipidemia) was preferentially selected to avoid overfitting the model. In instances where correlated medications and diseases both met criteria for inclusion in the model (eg, hyperlipidemia and statins), both the medication and disease were included in order account for alterations in physiology secondary to the disease as well as the pleotropic effects of the medication. ABI and CAD, which were utilized to classify our PAD and control groups, were collinear with PAD and were excluded from the multivariable model.

RESULTS

The overall cohort was predominantly male (97%) and Caucasian (71%) with an average age of 68±8 years old. Patients with PAD smoked more tobacco, were more likely to have hypertension and hyperlipidemia, and were more likely to use aspirin, beta-blockers, and statins (Table 1). Patients with PAD had a paradoxically lower LDL, which was likely due to the greater statin use observed in this group. Mean levels of hsCRP were similar between the two groups, which may be due to differences in medication use and/or similarities in comorbid diseases and pack year history.

Patients with PAD had a lower percent of erythrocyte EPA, DHA, and total n-3 PUFA relative to total fatty acid content (Table 2). Additionally, patients with PAD had a greater percent of erythrocyte total saturated fat; however, there were no differences in the percent of erythrocyte total trans fats or total monounsaturated fats. Patients with PAD also had a significantly lower omega-3 index (5.0±1.7% vs. 6.0±1.6%, $p<.001$) (Figure 1) and EPA:ARA ratio (Median, 0.03 [IQR 0.02 to 0.04] vs. 0.05 [IQR 0.03 to 0.07], $p<.001$). A sensitivity analysis that compared the omega-3 index between patients with PAD and controls after excluding patients with CAD, yielded similar results (4.8±1.5% vs. 6.0±1.6, $p<.001$). An additional secondary analysis of the PAD group only did not identify a statistically significant difference in the omega-3 index between patients with and without CAD (5.3±1.9% vs. 4.8±1.5, $p=.09$). These results collectively suggest that the difference in the omega-3 index observed between the PAD and control group was not entirely due to the difference in the prevalence of CAD.

In bivariate analyses, age, pack years smoked, eGFR, and several comorbidities and medications were significantly associated with PAD (Table 3). In a multivariable model including these patient characteristics, an absolute decrease of 1% in the omega-3 index was associated with 39% greater odds of PAD (OR: 1.39, 95%CI: 1.03 to 1.86, $p=.03$). A sensitivity analysis adjusting the multivariable model by smoking status instead of pack years yielded a similar result (OR: 1.46, 95%CI: 1.11 to 1.94, p=.008). Repeating the final model after excluding all patients with CAD resulted in similar findings (OR: 1.53, 95%CI: 1.09 to 2.17, $p=.02$). An additional sensitivity analysis completed in the PAD group that measured the association between the omega-3 index and CAD, did not result in a significant association (OR: 0.88, 95%CI: 0.68 to 1.15, $p=.35$).

DISCUSSION

To our knowledge, this is the first comparison of the omega-3 index in patients with and without PAD. In this cross-sectional analysis, we report that PAD is associated with a lower omega-3 index, independent of several patient characteristics, medications, and comorbidities. Additionally, patients with PAD had a lower EPA:ARA ratio and a greater mean total saturated fat erythrocyte membrane composition. Collectively, these results suggest that patients with PAD may not be consuming adequate amounts of n-3 PUFA or that they are unable to process and integrate n-3 PUFA into erythrocyte membranes as well as patients without PAD. As the incidence of PAD continues to rise, identification of risk factors associated with PAD will be key to reducing the impact of this disease.

Low plasma levels of n-3 PUFA have been associated with the presence and severity of coronary plaques (Urabe et al., 2013), progression of CAD (Erkkila et al., 2006), rate of adverse cardiac events (Itakura et al., 2011), and mortality (Masson et al., 2013). Similarly, a lower ratio of EPA to ARA in the plasma has been associated with higher prevalence of CAD (Tani et al., 2015) and acute coronary syndrome (Iwamatsu et al., 2016). The omega-3 index, which is likely a more reliable and consistent measure of n-3 PUFA tissue content (Harris et al., 2010), has been recognized as a marker of high cardiovascular risk and may actually measure a physiological phenomenon directly linked to the pathogenesis of cardiovascular disease and poor outcomes (Harris, 2009). Harris and colleagues recently

reported that a higher omega-3 index was independently associated with decreased cardiovascular and all-cause mortality in the community-based Framingham Heart Study (Harris et al., 2018). The association with all-cause mortality was also observed among patients with pre-existing CAD in the Heart and Soul Study (Pottala et al., 2010). Most available studies comparing risk stratify by quantiles of the omega-3 index (Harris et al., 2017) so it is not clear what the reported 1% difference equates to in regard to incidence of adverse cardiac events and cardiac death. The lack of a significant association with ARA levels per se indicates that the lower EPA to ARA ratio was due primarily to the lower EPA levels, not higher ARA levels. This is consistent with a lower omega-3 Index.

Studies of n-3 PUFA content in patients with PAD specifically are more limited. In a cross-sectional study of patients with pre-existing CAD, Suigara and colleagues reported that plasma EPA levels were significantly and independently lower among patients with PAD (Sugiura et al., 2014). In a cross-sectional analysis of 70 Japanese patients with atherosclerotic risk factors, a low plasma EPA to ARA ratio was independently associated with the presence of PAD (Fujihara et al., 2013). Gautam and colleagues also reported in a case-control analysis of patients in Japan that a low plasma EPA to ARA ratio was independently associated with PAD (Gautam et al., 2014). The same study also reported that patients with PAD have significantly lower levels of EPA and DHA, which is consistent with results of the current study. In this study, patients with PAD also had a lower level of the n-6 PUFA, γ-linolenic acid (18:3n-6), which was not observed in the current study. It is important to note that Gautam and colleagues measured levels of fatty acids in the plasma, whereas the current study measured the percent of fatty acids within the membranes of erythrocytes, a more stable marker of omega-3 status than plasma (Harris et al., 2010). Since the current study defined PAD as the presence of claudication symptoms and an ABI <0.9 or history of peripheral revascularization for claudication symptoms, regardless of ABI, some participants had a history of peripheral revascularization and likely had an abnormally elevated ABI. Therefore, analyses of correlation between ABI and individual lipids, or the omega-3 index, were not reported.

The associations observed between dietary intake and/or biomarker levels of n-3 PUFA and improved cardiovascular outcomes has prompted the exploration of the effects of supplementation with n-3 PUFA. Although results of these studies have varied, supplementation with n-3 PUFA has been reported to be associated with reductions in inflammation (He et al., 2009, Siasos et al., 2013), serum triacylglycerols (Backes et al., 2016), and cardiovascular risk (Harris et al., 2018). Although these beneficial effects have also been reported in patients with impaired renal function (Huang et al., 2013), it remains unclear whether the effect is modified by impaired renal function. There currently are minimal data on the effects that impaired renal function may have on PUFA metabolism and physiology. However, it has been reported that consumption of n-3 PUFA is beneficial in patients with chronic kidney disease and may reduce serum triacylglycerols, inflammation, and cardiovascular risk (Kim et al., 2018). Studies specific to patients with PAD are more limited, but report similar results (Grenon et al., 2012) in addition to increases in the omega-3 index and downstream lipid mediators of resolution of inflammation (Grenon et al., 2015). Variations in results of n-3 PUFA supplementation has been hypothesized to be due to differences in study samples and supplementation dosage and period (Burke et al., 2017).

The results of the current study report that patients with PAD are deficient in erythrocyte n-3 PUFA and the omega-3 index, suggesting a plausible mechanistic pathway to replenish, which may result in improved cardiovascular risk and reduced atherogenesis.

N-3 PUFA are thought to mediate their protective effects through several mechanisms including improvements in lipid profile, resting heart rate, blood pressure, vascular function, and increases in mediators of resolution of inflammation (Mozaffarian et al., 2011). Resolution of inflammation is characterized by active counter-regulation of inflammation and is a process driven by distinct mediators, including the specialized pro-resolving lipid mediators (SPM) (Serhan, 2014). SPM include the lipoxins, maresins, E-series and D-series resolvins, and protectins, all of which are downstream metabolic products of EPA, DHA, and ARA. SPM have been reported to regulate leukocyte-endothelial interactions (Spite et al., 2009), reduce the formation of reactive oxygen species (Nascimento-Silva et al., 2007), increase nitric oxide production (Paul-Clark et al., 2004), and have several protective effects on vascular cells (Chatterjee et al., 2014, Chattopadhyay et al., 2018, Ho et al., 2010, Miyahara et al., 2013), all of which have been hypothesized to be protective against atherosclerosis and vascular injury. Although clinical assessments of SPM in patients with PAD are limited, it has been reported that patients with PAD have a lower level of 15*R*-LXA_4, suggesting that patients with PAD might have a deficit of downstream products of PUFA metabolism (Ho et al., 2010). Additionally, Grenon and colleagues reported that the omega-3 index was inversely associated with systemic inflammation among patients with PAD (Grenon et al., 2013). Deficits in erythrocyte n-3 PUFA may represent a mechanism that may lead to deficiencies in SPM, and in turn, increased systemic and vascular wall inflammation, atherogenesis, and poor cardiovascular outcomes in patients with PAD.

The omega-3 index has been reported to be positively correlated with dietary intake of EPA and DHA (Harris et al., 2012) and results in increases in downstream SPM intermediates (Schaller et al., 2017). Previous studies have identified that patients with PAD have poor dietary intake of n-3 PUFA and other nutrients that may affect disease progression or outcomes (Nosova et al., 2015). The results of the current study confirm what previous assessments of dietary intake have suspected. Further prospective studies are needed to establish whether long-term n-3 PUFA supplementation or dietary management can modify the development and progression of PAD, and improve outcomes in patients with pre-existing PAD.

There are some limitations to this study. Since this is a cross-sectional study, conclusions on causality or directionality cannot be determined. All patients included in this study were Veterans recruited from the SFVAMC, mostly male, and Caucasian, thus the results of this study may not be generalizable to the general population.

In conclusion, we report that PAD is significantly and independently associated with a deficiency in erythrocyte n-3 PUFA and a lower omega-3 index. This deficiency identifies a plausible mechanism underlying the elevated systemic inflammation, poor response to vascular injury, and high cardiac risk that is observed among patients with PAD. Additionally, it identifies a potential therapeutic target that can be addressed with dietary intervention or with well-tolerated, low-risk fish oil supplementation. As the global

population ages and the prevalence of PAD grows, the ability to identify risk factors for PAD will become important in reducing morbidity and mortality associated with this disease.

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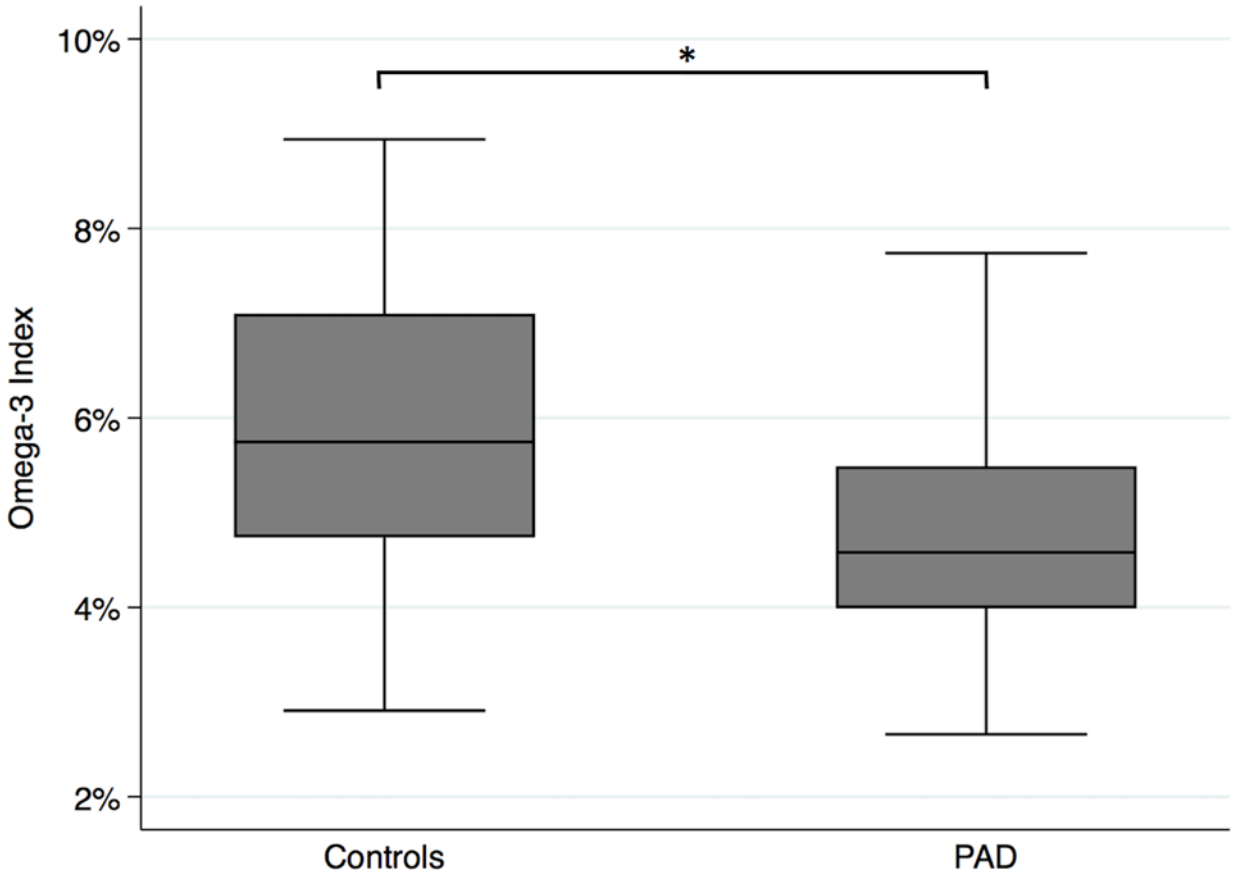

Fig. 1.

Patients with peripheral artery disease (PAD) have a significantly lower omega-3 index when compared to controls (Wilcoxon rank-sum test: $p<.001$)

Table 1.

Baseline Characteristics of Subjects with Peripheral Artery Disease and Controls

Characteristics	PAD (n=145)	Controls (n=34)	*P* value[a]
Age (years)	68.5 ± 7.5	67.6 ± 9.7	.56
Male sex	141 (97%)	33 (97%)	1.0
Caucasian	104 (72%)	23 (68%)	.67
BMI (kg/m^2)	28.1 ± 5.2	29.4 ± 4.1	.16
Comorbidities and Risk Factors			
Smoking Status			**.02**
Never	11 (8%)	6 (18%)	
Former	56 (39%)	5 (15%)	
Current	78 (54%)	22 (67%)	
Pack Years	43 ± 35	19 ± 23	**<.001**
ABI[b]	0.71 ± 0.16	1.13 ± 0.14	**<.001**
Hypertension	133 (92%)	23 (67%)	**.001**
Hyperlipidemia	125 (86%)	24 (71%)	**.04**
Type 2 diabetes mellitus	50 (34%)	6 (18%)	.07
Coronary artery disease[b]	59 (41%)	0 (0%)	**<.001**
Systolic blood pressure (mm Hg)	139 ± 19	134 ± 15	.16
Diastolic blood pressure (mm Hg)	77 ± 10	81 ± 9	**.04**
Medications			
Aspirin	104 (72%)	17 (50%)	**.02**
ACE-inhibitor	66 (46%)	13 (38%)	.57
Beta-blocker	82 (57%)	8 (24%)	**.001**
Statin	121 (83%)	20 (59%)	**.004**
Insulin	18 (12%)	1 (3%)	.13
Oral diabetes medications	38 (26%)	4 (12%)	.11
Laboratory Studies			
Omega-3 index (%)	5.0 ± 1.7	6.0 ± 1.6	**<.001**
Total Cholesterol (mg/dL)	162 ± 41	173 ± 36	.16
LDL (mg/dL)	87 ± 35	102 ± 30	**.02**
HDL (mg/dL)	46 ± 14	47 ± 11	.58
Triacylglycerols (mg/dL)	149 ± 95	126 ± 104	.08
eGFR (mL/min)	74.7 ± 22.8	85.5 ± 22.9	**.02**
Albumin (g/dL)	4.0 ± 0.3	4.1 ± 0.3	.40
HbA1C (%)	6.2 ± 1.3	5.9 ± 1.4	.24
hsCRP (mg/L)	4.2 ± 4.2	4.4 ± 4.8	.96

Values as “means±SD” or “n (%)”. Boldface *P* values were below the .05 level required for statistical significance. ABI = ankle-brachial index; ACE = angiotensin-converting enzyme; BMI = body mass index; eGFR = estimated glomerular filtrate rate; HDL = high-density lipoprotein; HbA1c = hemoglobin A1c; hsCRP = high-sensitivity C-reactive protein; LDL = low density lipoprotein

[a]Calculated using a Fisher’s exact test for categorical variables, two-tailed Students *t*-test for normally distributed continuous variables, and a Wilcoxon rank-sum test for continuous variables without a normal distribution (omega-3 index, pack years, hsCRP, and triacylglycerols).

[b] An ankle-brachial index >0.9 and the absence of coronary artery disease were both selection criteria for control patients.

Table 2.

Constituent Fatty Acids of the Erythrocyte Membrane in Subjects with Peripheral Artery Disease and Controls

	PAD (n=145)		Controls (n=34)		*P* value[a]
Fatty acid (mole %)	**Median**	**IQR**	**Median**	**IQR**	
n-3 Fatty acids, total	6.00	5.21–7.06	7.10	5.92–8.01	**.03**
18:2n-3	0.10	0.10–0.21	0.21	0.10–0.31	.15
20:5n-3	0.48	0.38–0.67	0.67	0.48–0.96	**<.001**
22:5n-3	2.18	1.66–2.53	2.04	1.50–2.57	.73
22:6n-3	3.25	2.72–4.04	4.18	3.21–4.63	**.02**
n-6 Fatty acids, total	34.45	32.73–35.59	33.48	31.25–34.78	.98
18:2n-6	11.74	9.90–13.98	12.84	10.77–19.05	.10
18:3n-6	0.10	0.10–0.21	0.21	0.10–0.31	.20
20:3n-6	0.28	0.23–0.29	0.28	0.25–0.28	.24
20:3n-6	1.59	1.31–1.78	1.52	1.43–1.81	.61
20:4n-6	16.04	13.50–17.18	14.75	12.26–16.85	.12
22:4n-6	3.99	2.00–4.51	3.35	1.67–4.40	**.03**
22:5n-6	0.70	0.52–0.87	0.53	0.44–0.62	**.002**
Saturated fat, total	43.13	41.74–43.88	42.59	38.40–43.45	**.01**
14:0	0.50	0.32–0.52	0.38	0.34–0.54	.88
16:0	24.12	23.23–25.12	23.69	23.01–24.70	**.02**
18:0	17.93	16.32–18.53	17.78	12.67–18.29	.09
20:0	0.13	0.11–0.14	0.15	0.13–0.19	**<.001**
22:0	0.14	0.10–0.22	0.20	0.15–0.35	**.001**
24:0	0.31	0.16–0.47	0.40	0.32–0.80	**<.001**
Trans fat, total	1.26	1.05–1.58	1.09	0.98–1.41	.16
16:1n-7t	0.15	0.12–0.18	0.16	0.14–0.17	.88
18:1t	0.81	0.61–1.01	0.72	0.62–0.93	.29
18:2n-6t	0.31	0.22–0.33	0.21	0.21–0.31	**.04**
Monounsaturated fat, total	15.16	14.25–17.68	15.74	14.24–19.26	.39
16:1n-7	0.34	0.22–0.56	0.34	0.23–0.68	.85
18:1n-9	14.30	13.49–16.43	14.72	13.69–18.11	.61
20:1n-9	0.20	0.18–0.23	0.21	0.19–0.24	.15
24:1n-9	0.32	0.24–0.47	0.48	0.32–0.80	**<.001**

Boldface *P* values were below the .05 level required for statistical significance.

[a]Calculated using a Wilcoxon rank-sum test.

Table 3.

Bivariate and Multivariable Associations Between the Omega-3 index and Peripheral Artery Disease (n=179)

	Bivariate Analysis			Multivariable Analysis[a]		
Predictor	**OR**	**95% CI**	***P* value**	**OR**	**95% CI**	***P* value**
Omega-3 index (per 1-unit decrease)[b]	1.34	1.09–1.64	.005	1.39	1.03–1.86	**.03**
Age (years)	1.04	0.98–1.09	.17	1.00	0.93–1.08	.95
Pack years	1.03	1.01–1.06	.001	1.04	1.01–1.06	**.002**
eGFR	0.98	0.96–0.99	.02	0.98	0.96–1.01	.17
Hypertension	6.84	2.54–18.37	<.001	2.36	0.62–9.01	.21
Hyperlipidemia	2.77	1.12–6.86	.03	1.01	0.23–4.31	.99
Type 2 diabetes mellitus	2.65	1.00–7.05	.05	0.32	0.03–3.39	.34
Aspirin	3.22	1.42–7.29	.005	3.39	1.20–9.54	**.02**
Statin	3.93	1.69–9.15	.001	1.48	0.38–5.74	.57
Beta-blocker	4.96	2.00–12.31	.001	3.66	1.29–10.42	**.02**
Insulin	5.38	0.64–44.98	.12	3.79	0.21–68.33	.37
Oral diabetes medications	3.32	1.03–10.72	.04	4.72	0.39–56.34	.22

Boldface *P* values were below the .05 level required for statistical significance in the multivariable model. CI = confidence interval; eGFR = estimated glomerular filtration rate; OR = odds ratio.

[a]The bivariate logistic regression model coefficient estimates the associated OR (95% CI) of peripheral artery disease per 1-unit change in the predictor when controlling for the omega-3 index. All variables from Table 1 were run through a bivariate analysis to screen for potential confounders. All variables with a *P* value < .20 in the bivariate analysis were included in the multivariable model.

[b]Omega-3 index was run as a univariate logistic regression because it was the predictor in the bivariate analysis.