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Clinical correlates and heritability of erythrocyte eicosapentaenoic and docosahexaenoic acid content in the Framingham Heart Study

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Abstract

Objectives—Red blood cell (RBC) levels of eicosapentaenoic acid (EPA) plus docosahexaenoic acid (DHA, the omega-3 index, expressed as a percent of total fatty acids) are inversely related to risk for cardiovascular disease (CVD). Although several mechanisms underlying this relationship have been proposed, understanding the associations between the omega-3 index and markers of CVD in the community can shed additional light on this question. The objectives of this study were to define the relations between the omega-3 index and clinical factors and to determine the heritability of the omega-3 index.

Methods—RBC samples (n = 3196) drawn between 2005 and 2008 from participants in the Framingham Study [Examination 8 of the Offspring cohort plus Examination 3 of the Omni (minorities) cohort] were analyzed for fatty acid composition by gas chromatography.

Results—The mean (SD) omega-3 index was 5.6% (1.7%). In multivariable regression models, the factors significantly and directly associated with the omega-3 index were age, female sex,

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Potential conflicts of interest

WSH is a scientific advisor to companies with interests in fatty acids including Monsanto, Aker Biomarine, Omthera, Amarin and GlaxoSmithKline, and was a speaker for the latter. In addition, he is the owner of OmegaQuant Analytics, LLC and an employee of Health Diagnostics Laboratory, Inc., both of which offer blood fatty acid testing commercially. None of the other authors have any potential conflicts to disclose. All authors had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Appendix A. Supplementary data

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.atherosclerosis.2012.05.030.

higher education, fish oil supplementation, dietary intake of EPA + DHA, aspirin use, lipid pharmacotherapy, and LDL-cholesterol. Factors inversely associated were Offspring cohort, heart rate, waist girth, triglycerides and smoking. The total explained variability in the omega-3 index for the fully adjusted model was 73%, which included major components due to heritability (24%), EPA + DHA intake (25%), and fish oil supplementation (15%).

Conclusion—The variability in the omega-3 index is determined primarily by dietary and genetic factors. An increased omega-3 index is associated with a generally cardioprotective risk factor *milieu*.

Keywords

Epidemiology; Cardiovascular disease; Risk factors; Omega-3 fatty acids; Erythrocytes; Heritability

1. Introduction

Red blood cell (RBC) levels of the long chain marine omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which together constitute the omega-3 index [1], have been reported to be inversely related to risk for sudden cardiac death [2,3], acute coronary syndromes [4], depression [5], and total mortality [6] as summarized in recent reviews [7-9]. These may be secondary to effects of these fatty acids on heart rate [10], inflammation [11], endothelial function [12], serum lipid levels [13] and/or platelet function [14], which themselves may be mediated by changes in eicosanoid metabolism [15], membrane biophysics [16], and gene expression [17]. Although n-3 fatty acid supplementation has been successful in reducing risk for CVD events in several large randomized trials [18], more recent experience has been less uniform [19]. Indeed, the proposed anti-arrhythmic mechanism has recently been questioned [20].

An examination of the relations between RBC omega-3 content and markers of cardiovascular disease (CVD) in the community (where fish oil supplementation is uncommon) can help generate hypotheses regarding mediating mechanisms, whereas the associations of demographic and lifestyle factors with the omega-3 index can help define the clinical and behavioral variables that determine its levels. Among the latter are obviously fish intake and fish oil supplementation, but the effects of age, sex, smoking status, etc. and of genetic factors on the omega-3 index are unclear. The purpose of this study was to examine these cross-sectional relations in a well-characterized community, the Framingham Offspring cohort.

The red blood cell (RBC) membrane has been used by several investigators to quantify relatively long-term dietary exposure to *trans* and marine n-3 dietary fatty acids [3,21,22]. Indeed, fatty acid biomarkers (in plasma, plasma phospholipids, RBCs, etc) are better predictors of incident congestive heart failure [23], atrial fibrillation [24] and type 2 diabetes mellitus [25] than are questionnaire-based intake estimates. Hence n-3 fatty acid biomarkers are superior to n- 3 fatty acid intake estimates for predicting disease outcomes.

2. Methods

2.1. Participants

Children (and their spouses) of the original Framingham Heart Study cohort were recruited in 1971 and constitute the Framingham Offspring Study [26]. The detailed study designs and methods have been extensively described (http://www.nhlbi.nih.gov/about/framingham). In 1994, recruitment began for the Framingham Omni cohort comprising residents aged 40–74 who described themselves as members of a minority group, [27]. The Offspring Exam 8 and

Omni Exam 3 were scheduled together from 2005 to 2008, and totaled 3319 participants. All study participants were comprehensively evaluated in an examination that included anthropometric measurements, biochemical assessment for traditional CVD risk factors, medical history and physical examination by a study physician. Written informed consent was provided by all participants, and the Institutional Review Board at the Boston University Medical Center approved the study protocol.

2.2. RBC fatty acid analysis

RBCs were isolated from blood drawn after a 10–12 h fast and frozen at −80 °C immediately after collection. RBC fatty acid composition was analyzed by gas chromatography (GC) with flame ionization detection as previously described [56]. Briefly, unwashed, packed RBCs were directly methylated with boron trifluoride and hexane at 100 °C for 10 min. The fatty acid methyl esters thus generated were analyzed using a GC2010 Gas Chromatograph (Shimadzu Corporation, Columbia, MD) equipped with an SP2560, fused silica capillary column (Supelco, Bellefonte, PA). Fatty acids were identified by comparison with a standard mixture of fatty acids characteristic of RBC (GLC 727, NuCheck Prep, Elysian, MN) which was also used to determine individual fatty acid response factors. The omega-3 index is defined as the sum of EPA and DHA expressed as a percent of total identified fatty acids. The coefficients of variation were 6.2% for EPA, 4.4% for DHA and 3.2% for the omega-3 index. All fatty acids present at >1% abundance had CVs of ≤7%.

2.3. Diet assessment

N-3 fatty acid intakes at Offspring Exam 8 were estimated by food frequency questionnaire, FFQ [28]. (No FFQ data were available from Omni Exam 3). Validity of this instrument for fatty acid intake has been documented by comparison with adipose tissue fatty acid composition [29,30] where the correlation for marine n-3 fatty acids was 0.43 ($p < 0.0001$). A nutrient database (released in 2002) was used to calculate the fatty acid composition of diets. Fish oil supplementation use was determined by self-report under Medications as “fish oil” or “omega-3” but not “flaxseed oil.”

2.4. Statistical analysis

There were 3196 participants from Offspring ($n = 2899$) and Omni ($n = 297$) with fatty acid data available for analyses. Differences in clinical factors, demographics and individual fatty acids between cohorts were tested using *t*-tests and chi-squared tests for continuous and categorical data, respectively.

There were 2964 subjects with a complete set of covariates (except for dietary data) for analyses. The n-3 fatty acids were transformed using natural logarithm to improve normality and homoscedasticity of the residuals in the adjusted linear models. The primary analysis examined the relations between ln (EPA + DHA) (dependent variable) with clinical factors (predictor variables). Age, sex, cohort, and highest level of education attained (the later as a marker of socioeconomic status) were forced into all models. Additionally PROC GLMSELECT was used with stepwise variable selection and Schwarz Bayesian Criterion [31] to select the ‘best’ model [32] from 21 candidate variables: systolic and diastolic blood pressure, heart rate, treatment for hypertension, waist girth at umbilicus, alcohol intake (heavy drinking defined as >14 drinks per week for men and >7 for women), physical activity index [33], current smoking, LDL- and HDL-cholesterol, triglycerides, fish oil supplementation, lipid lowering treatment, glucose, hemoglobin A1c, urinary albumin/creatinine, aspirin use, and prevalent diabetes, cardiovascular disease, congestive heart failure, and coronary heart disease. Triglycerides, physical activity index, and urinary albumin/creatinine were log-transformed to reduce leverage of extreme values. Then the

selected model was refitted using generalized least squares in PROC MIXED which included a random coefficient to incorporate the familial clustering among siblings (Supplementary Table 1). The statistical parameters were estimated using restricted maximum likelihood with the 'sandwich' variance estimator; these standard errors are robust to misspecification of the covariance structure [34]. Covariates in the final model were collectively tested for interactions with age or sex using the partial F-test. In a secondary analysis, the individual RBC n-3 fatty acids (i.e., alpha-linolenic acid (ALA), docosapentaenoic acid (DPA), EPA and DHA) were modeled using the above method. For all results the critical level alpha was set to 0.05/25 = 0.0020 for statistical significance using Bonferroni correction.

To determine the extent to which the intake of EPA + DHA may have mediated the relations between the omega-3 index and the selected covariates, a subgroup analysis was conducted in the 2182 Offspring participants with dietary information. Bootstrapping was used with 1000 iterations to build nonparametric confidence intervals of the difference in the regression coefficients [35-37] between models that included or did not include dietary intake. Since 14 variables were tested for dietary mediation, the critical level was controlled using Bonferroni correction at 0.05/14 tests = 0.0036 (i.e., 99.64% confidence intervals). Clinical correlates analyses were performed using SAS software (version 9.2; SAS Institute Inc., Cary, NC).

The Offspring cohort with dietary intake (N = 2182) was used for heritability analyses of ln (EPA + DHA) since pedigree data were not collected in Omni. The same final multivariable model was used as above, which included – age, sex, highest education, heart rate, waist girth at umbilicus, current smoking, LDL and HDL cholesterol, ln[triglycerides], lipid lowering treatment, ln[n-3 dietary intake], fish oil supplementation and aspirin use. Unrelated participants were excluded, resulting in 2030 for analyses. Heritability estimates were calculated using the polygenic function in SOLAR applied to residuals from the adjusted linear models [38]. The initial model adjusted for age and sex; fish oil, ln[n-3 dietary intake], and smoking were added individually and the change in explained variance was determined. The fully adjusted model included the covariates listed above. We used the robust multivariate t-distribution, which includes a weighting parameter to reduce the potential effect of skewed residuals [39].

3. Results

3.1. Clinical correlates

There were several differences between the Offspring (white) and Omni (non-white) cohorts as seen in Table 1. The Offspring were on average 4 years older, less educated, and had a lower prevalence of diabetes. They also had higher levels of alcohol consumption, smoking, physical activity and fish oil supplementation. The overall mean (SD) omega-3 index was 5.62% (1.71%). It was 5.56% (1.69%) in the Offspring, but 6.16% (1.83%) in Omni (p < 0.0001) despite fewer Omni participants taking fish oil supplements. The difference was due primarily to a higher DHA level (Supplemental Table 2). A higher omega-3 index was found in non-Hispanic African American (n = 123) and Asian (n = 74) participants in whom the mean was 6.4%; in the Omni Hispanics (n = 80), the index (5.6%) was similar to that of the Offspring cohort. Whether nutritional factors were responsible for these differences could not be determined since dietary information was not available for Omni.

Of 22 clinical variables examined individually, nine were significantly associated with the index (Table 2). Those directly related were the use of fish oil, aspirin, or lipid pharmacotherapy, whereas diastolic blood pressure, heart rate, waist girth, BMI, current smoking, and serum triglyceride levels were inversely related. Adjusted linear models were

constructed to examine the relations between the omega-3 index with clinical and demographic factors (Table 3). The Pearson correlation between BMI and waist girth was very strong ($r = 0.91$), so BMI was excluded from multivariable models. In this analysis, factors directly associated with the omega-3 index were age, female sex, higher education, fish oil supplementation, aspirin use and/or lipid pharmacotherapy, and LDL-cholesterol. Factors inversely related included Offspring cohort, heart rate, waist girth, triglycerides and smoking. The relations between EPA + DHA and the variables in the adjusted model were not modified by gender ($p = 0.27$) or age ($p = 0.19$). In general, the same relations were observed between these factors and the individual fatty acids (EPA and DHA) as with the sum of the two (cf. Table 3 and Supplementary Table 3). The primary exception was for HDL-cholesterol which was not selected into the omega-3 index model (Table 3), because the significant relations with EPA (direct) and DHA (inverse) canceled each other (Supplementary Table 3). For the other two n-3 fatty acids, DPA and ALA, far fewer variables were selected into their models (Supplementary Table 4) than into the index model. Of note, DPA was lower in women than men, whereas DHA was higher. Also, compared to EPA and DHA (which had inverse relations with triglycerides), ALA was directly related to triglycerides.

3.2. Effects of Diet

Dietary intake of EPA + DHA was available for 2182 Offspring cohort participants, so the question of the extent to which differences in dietary intake of n-3 fatty acids mediated the associations between the omega-3 index and the clinical/demographic factors was addressed in this subset (Supplementary Table 5). The EPA + DHA intake attenuated the associations between the omega-3 index and two factors: fish oil supplementation and education. Without EPA + DHA intake in the model, fish oil supplementation (yes/no) was associated with 36% [column 1; exp(0.311) = 1.36] higher levels of EPA + DHA. This, however, was an overestimate because those who took fish oil supplements also choose to eat more fish. A less biased estimate of the effect of fish oil supplementation alone, taking into account the coincidently higher EPA + DHA intake from the diet, was 19% [column 4; exp(0.178) = 1.19]. Likewise, the 12% higher omega-3 index level associated with having a graduate-level degree [column 1; exp(0.109) = 1.12] was more appropriately estimated as about 6% higher [column 4; exp(0.057) = 1.06] since highly educated participants also ate more fish. Importantly, relations between the omega-3 index and other demographic or clinical factors were not mediated by diet, so the entire sample was used to generate unbiased parameter estimates (Table 3). The correlations between the intake of EPA + DHA with RBC levels of these fatty acids in participants not taking fish oil supplements ($n = 1949$) was 0.59 ($p < 0.0001$); and was 0.19 ($p = 0.003$) in those taking supplements ($n = 233$).

3.3. Heritability

Five statistical models with increasing levels of complexity were used to partition the variance in the omega-3 index into that determined by the model covariates (those shown in Supplementary Table 5) and that determined by heritability, the later being based on pedigree information (Fig. 1). With age and sex as the only covariates, heritability explained 50% of the variability in the omega-3 index. When fish oil supplementation was added as a covariate to the model, the variance explained by the covariates increased from 4% to 19%, but there was little change in the heritability estimate (from 50% to 47%). When dietary ln(EPA + DHA) intake was next added to the model, the covariates' variance estimate increased to 44%, and the heritability estimate decreased to 32%. Further covariate adjustment produced minimal changes. The total explained variability in the omega-3 index for the fully adjusted model was 73%, which included major components due to heritability (24%), dietary intake (25%), and fish oil supplementation (15%).

4. Discussion

The purpose of this study was to explore the clinical, demographic, behavioral and genetic correlates of the omega-3 index in a well-defined cohort of subjects from the Framingham Heart Study. The cohort was comprised of 91% white participants (Offspring) and 9% minorities (Omni). The omega-3 index was lower in Offspring than Omni despite fish oil supplementation being more prevalent in the former; this effect remained after multivariable adjustment. Whether a higher dietary intake of n-3 fatty acids in Omni is responsible for this difference is unknown since no dietary information was available for this cohort. Higher levels of the omega-3 index in African Americans compared with Whites have been reported in acute myocardial infarction patients [40].

Overall the omega-3 index was directly associated with several factors in fully adjusted models. A significant relationship with age has been frequently reported [41-51] and could reflect increased fish intake in older individuals. However, we observed this association after controlling for n-3 fatty acid intake [as have some [42,44,46,52] but not all [53,54] other studies], and thus it may be a function of decreased n-3 fatty acid turnover in older subjects [55]. In an earlier study of 298 Offspring participants with RBCs tested 6.7 years apart [56], the mean omega-3 index did not change, suggesting that higher levels of the index in older people may not be due to aging per se, but perhaps to the “attrition of the susceptible.” Also female sex was associated with a higher omega-3 index. This too has been reported previously [48,49,53,57,58] [but inconsistently [42-44,50,51]], and could be due to enhanced synthesis of long chain n-3 fatty acids from alpha-linolenic acid mediated by estrogen [59,60]. This explanation is tentative, however, since the vast majority of women in this cohort were post menopausal, and only 10% were on hormone therapy. The reduced DPA (intermediate between EPA and DHA) and increased DHA levels in post menopausal women vs. men observed here are consistent with increased conversion of DPA to DHA seen in premenopausal [61] women. A higher omega-3 index was also directly associated with a greater use of aspirin and lipid lowering drugs, and with higher LDL-C levels. This confirms smaller crosssectional observations made in Canadian native Americans [50] and Inuits [49]. In the present study, the people taking aspirin and/or lipid drugs were more likely to be taking fish oil supplements (58% vs. 42%, $p < 0.0001$). In addition, they may have been taking higher n-3 fatty acid doses than the supplementers not on pharmacotherapy (dosage amounts were not available) which could explain the association of higher omega-3 index levels with medication use even after adjustment.

As regards the direct association between plasma LDL-C and the omega-3 index, n-3 fatty acid supplementation has been reported to increase LDL-C, particularly in hypertriglyceridemic patients [62], but this may be due more to a change in particle composition than in particle number [63]. Whether nutritional variations in n-3 fatty acid intakes affect LDL-C is not known, but the relation between the omega-3 index and LDL-C became non-significant when dietary EPA + DHA was included in the model (Supplementary Table 5).

There were several inverse associations with the omega-3 index in the fully adjusted model. That with smoking status, which has been noted before [40-43,48-50,53,64], was driven by the DHA (not the EPA) component (Supplementary Table 3). This relationship may be due to the oxidative destruction of most highly unsaturated fatty acids [65,66], and/or from altered metabolism of EPA and DHA in smokers. The fact that smoking was associated with lower omega-3 levels independent of EPA + DHA intake and fish oil supplementation (Supplementary Table 5) argues against intake differences.

Waist circumference was inversely associated with the omega-3 index (and both of its fatty acid components) in univariate and multivariable models. Studies in three Canadian cohorts reported contradictory findings in this regard: inverse associations between waist girth and RBC n-3 fatty acids in Cree Indians [50] but direct relations in Inuits [49] and Quebecers [48]. Therewas no univariate relation with girth in a Spanish cohort [53]. In a previous study, lower levels of EPA + DHA were associated with higher levels of pro-oxidants in RBCs from obese subjects [67], and with higher levels of inflammatory markers [43,68,69] suggesting that increased adiposity, oxidative stress, and inflammation and lower membrane n-3 fatty acids appear to coexist.

The triglyceride-lowering effects of supplemental n-3 fatty acids are well-known [13]. However, relations between triglycerides and n-3 fatty acid biomarkers in (largely) non-supplementing populations have received less attention. Inverse associations were, however, observed in the Heart and Soul study [70], Canadian Inuits [49], Spanish patients at high CHD risk [53] and outpatients in Kansas City [42]. These are likely to be causal since n-3 fatty acids lower TG production in the liver [71]. Finally, a higher omega-3 index was associated with a lower heart rate. This too has been reported in intervention studies [72,73] and observed crosssectionally [10,74]. N-3 fatty acids appear to improve autonomic tone as evidenced by their ability to increase heart rate variability in some but not all studies [75].

Taking advantage of the pedigree information from the Framingham study, we were able to estimate that 24% of the variability in the omega-3 index was due to polygenic factors (i.e., heritability). This increased the total explained variability to 73%, which was greater than previously reported [42,76]. The largest shift in amount of explained variance between heritability (15% decrease) and covariates (25% increase) was observed after dietary EPA + DHA intake was included in the model. That is to say, that related participants apparently maintained similar eating habits, and that the variance in the omega-3 index attributed to genes was overestimated when EPA + DHA consumption was not taken into account. Genome-wide association studies are needed to identify genetic loci that may determine the observed heritability [77].

In the Offspring cohort (where dietary data were available), the correlation between EPA + DHA intake and the omega-3 index (0.59) was stronger than that observed previously using the same FFQ but with adipose tissue EPA + DHA as a biomarker ($r = 0.43$) [30]. As RBC membranes are more enriched in these long chain n-3 fatty acids than is adipose tissue [78], this finding provides stronger confirmation of the validity of the FFQ for these specific fatty acids.

4.1. Strengths and limitations

Among the strengths of this study were the large, well-characterized, and racially-diverse (via the inclusion of Omni) cohort; and the use of validated analytical methods for clinical CVD risk markers (including the omega-3 index). Also this study included a formal analysis of heritability. There were also limitations. Causal relations cannot be deduced from a cross-sectional analysis, so the associations observed here – unless previously observed in n-3 fatty acid intervention studies – must be considered only hypothesis-generating. For those individuals reporting fish oil supplementation we had no information on the dosage, potency or intake frequency of the capsules. As noted earlier, we did not have data on the dietary habits nor pedigree information of the Omni participants.

In summary, a higher omega-3 index was independently associated with some factors known to be associated with reduced risk for cardiovascular disease (female sex, smaller waist girth, lower triglyceride levels, less smoking, lower heart rate, and a greater use of aspirin and lipid pharmacotherapy). On the other hand, a higher index was also associated with a

slightly higher LDL-cholesterol level. After adjusting for n-3 fatty acid intake and other relevant covariates, 24% of the variability in the omega-3 index was explained by Mendelian inheritance. Overall, an increased omega-3 index appears to coexist with a healthier cardiovascular risk profile.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Roles of authors

WSH, SJR, RSV, and MGL designed research (project conception, development of overall research plan, and study oversight); WSH conducted research (oversaw the hands-on lab analyses); JVP and SML (with oversight from MGL) analyzed data or performed statistical analysis; WSH and JVP wrote paper with major editorial input from SJR, MGL and RSV; and WSH had primary for final content. All authors read and approved the final manuscript.

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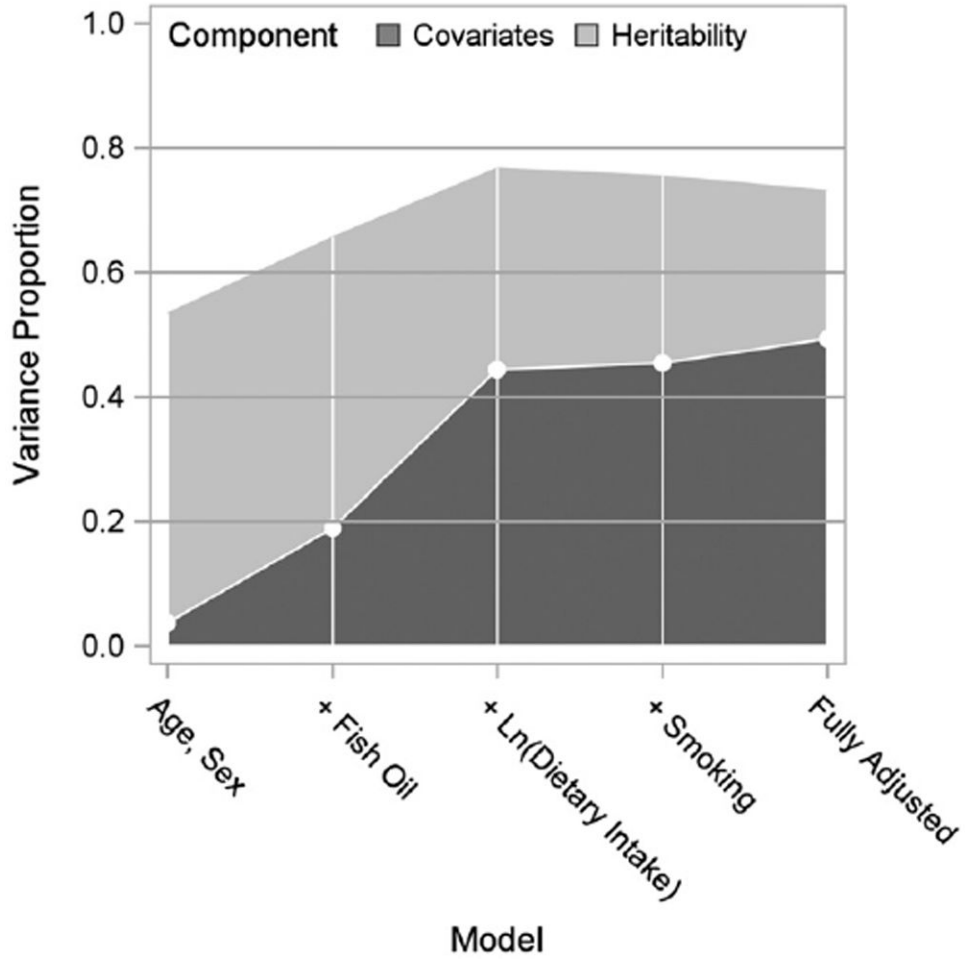

Fig. 1.

Total variance explained in the omega-3 index (RBC EPA + DHA) partitioned by heritability and covariates components for increasing model complexity. The fully adjusted model also included: HDL-C, LDL-C, ln(triglycerides), waist girth, aspirin (3+/wk), heart rate, lipid pharmacotherapy, and highest level of education.

Table 1

Offspring (Exam 8) and Omni (Exam 3) subjects' characteristics.

Variable [% missing][a]	All *N* = 3196	Offspring *N* = 2899	Omni *N* = 297	*P*-value[b]
Demographics				
Age (years)	66(9)[c]	66(9)	62(9)	**<0.0001**
Female	1762(55%)	1577(54%)	185(62%)	0.0092
Highest education				**<0.0001**
Grades <12	136(4%)	103(4%)	33(11%)	
Grade 12	1485(47%)	1411(49%)	74(26%)	
Associate/bachelor	927(29%)	840(29%)	87(30%)	
Graduate or professional	617(19%)	522(18%)	95(33%)	
Clinical factors				
Systolic (mm Hg)	128(17)	129(17)	127(19)	0.24
Diastolic (mm Hg)	74(10)	73(10)	74(9)	0.18
Heart rate (bpm)	62(10)	62(10)	61(10)	0.048
Hypertension treatment	1566(49%)	1408(49%)	158(53%)	0.13
Waist girth at umbilicus (inches)	40.0(5.8)	40.0(5.7)	39.7(6.3)	0.30
BMI (kg/m^2)	28.4(5.5)	28.3(5.4)	29.0(6.4)	0.029
1–2 drinks/day[d] [1.3%]	414(13%)	404(14%)	10(3%)	**<0.0001**
Physical activity index [1.1%]	35.2(5.3)	35.3(5.4)	34.2(4.6)	**0.0014**
Current smoker	270(8%)	259(9%)	11(4%)	**0.0020**
LDL-cholesterol (mg/dL)	105(32)	105(31)	106(34)	0.54
HDL cholesterol (mg/dL)	57(18)	57(18)	57(17)	0.91
Triglycerides (mg/dL)	118(71)	118(69)	115(83)	0.48
Lipid pharmacotherapy	1373(43%)	1246(43%)	127(43%)	0.92
Fish oil supplementation	322(10%)	308(11%)	14(5%)	**0.0013**
Aspirin (3+/week)	1383(43%)	1278(44%)	105(35%)	0.0035
Glucose (mg/dL)	107(24)	107(24)	107(27)	0.78
Hemoglobin A1c %	5.8(0.7)	5.7(0.7)	6.0(0.8)	**<0.0001**
Diabetes	462(14%)	398(14%)	64(22%)	**0.0003**
Urine albumin/creatinine (mg/g) [1.7%]	0.26(1.29)	0.26(1.32)	0.23(1.02)	0.74
Prevalent cardiovascular disease	498(16%)	464(16%)	34(11%)	0.039
Prevalent congestive heart failure	77(2%)	75(3%)	2(1%)	0.041
Prevalent coronary heart disease	326(10%)	307(11%)	19(6%)	0.023

[a] All variables had <1% missing data unless noted otherwise.

[b] For testing differences between the two cohorts the critical level alpha was set to 0.05/25 factors = 0.0020 for statistical significance using Bonferroni correction (shown in bold).

[c] Mean (SD).

[d] Indicator variable for females > 7 drinks/week, males > 14 drinks/week.

Table 2

Clinical correlates of RBC EPA + DHA in individual models.

Variable (*N* = 2964)	Proportional change[a]	95% CI		*P*-value[b]
		Lower	Upper	
Blood pressure				
Systolic (1 SD = 17 mm Hg)	1.00	0.99	1.01	0.73
Diastolic (1 SD = 10 mm Hg)	0.98	0.97	0.99	**0.0018**
Heart rate (1 SD = 10 bpm)	0.96	0.95	0.97	**<0.0001**
Hypertension treatment	1.03	1.01	1.06	0.0033
Anthropometric & behavioral				
Waist girth (1 SD = 5.8 in)	0.97	0.96	0.98	**<0.0001**
BMI (1 SD = 5.5 kg/m^2)	0.97	0.96	0.98	**<0.0001**
1 – 2 Drinks/Day[c]	0.98	0.95	1.02	0.31
ln[Physical activity index] (1 SD)	1.00	0.99	1.01	0.97
Current smoker	0.84	0.81	0.87	**<0.0001**
Lipids & drugs				
LDL-cholesterol (1 SD = 32 mg/dL)	0.99	0.98	1.00	0.14
HDL cholesterol (1 SD = 18 mg/dL)	1.01	1.00	1.02	0.051
ln[Triglycerides] (1 SD)	0.97	0.96	0.98	**<0.0001**
Lipid pharmacotherapy	1.08	1.05	1.10	**<0.0001**
Fish oil supplementation	1.38	1.33	1.43	**<0.0001**
Aspirin (3+/week)	1.08	1.06	1.10	**<0.0001**
Glycemic control				
Glucose (1 SD = 23 mg/dL)	0.99	0.98	1.00	0.18
Hemoglobin A1c (1 SD = 0.7%)	1.00	0.98	1.01	0.37
Diabetes	0.98	0.95	1.01	0.15
ln[Urine albumin/creatinine] (1 SD)	1.00	0.99	1.01	0.59
Cardiovascular disease				
Prevalent cardiovascular disease	1.03	1.00	1.06	0.074
Prevalent congestive heart failure	1.00	0.93	1.08	0.98
Prevalent coronary heart disease	1.05	1.01	1.09	0.0080

[a] Represents the proportional change in EPA + DHA per 1 SD (continuous) or presence vs. absence (categorical) covariate.

[b] The critical level alpha was set to 0.05/25 factors = 0.0020 for statistical significance using Bonferroni correction (shown in bold).

[c] Indicator variable for females > 7 drinks/week, males > 14 drinks/week.

Table 3

Clinical correlates of RBC EPA + DHA in multivariable model.

Variable (*N* = 2964)	Proportional change[a]	95% CI		*P*-value[b]
		Lower	Upper	
Demographics				
Age (10 years)[c]	1.05	1.04	1.06	**<0.0001**
Sex (female)[c]	1.04	1.02	1.07	**<0.0001**
Cohort (offspring)[c]	0.89	0.85	0.92	**<0.0001**
Highest education[c]				
Graduate or professional	1.12	1.08	1.15	**<0.0001**
Associate/bachelor	1.09	1.06	1.11	**<0.0001**
Grade 12 (reference)	1.00	–	–	–
Grades <12	0.93	0.88	0.97	**0.0020**
Blood pressure				
Heart rate (1 SD = 10 bpm)	0.98	0.97	0.99	**<0.0001**
Anthropometric & behavioral				
Waist girth (1 SD = 5.8 in)	0.98	0.97	0.99	**0.0003**
Current smoker	0.90	0.87	0.93	**<0.0001**
Lipids & drugs				
LDL-cholesterol (1 SD = 32 mg/dL)	1.02	1.01	1.03	**0.0009**
Ln[Triglycerides] (1 SD)	0.98	0.97	0.99	**<0.0001**
Lipid pharmacotherapy	1.07	1.04	1.09	**<0.0001**
Fish oil supplementation	1.35	1.31	1.39	**<0.0001**
Aspirin (3+/week)	1.04	1.02	1.06	**0.0005**

[a] Represents the proportional change in EPA + DHA per 1 SD (continuous) or presence vs. absence (categorical) covariate, e.g. a 1 SD increase in Waist girth is associated with 2% lower levels.

[b] The critical level alpha was set to 0.05/25 factors = 0.0020 for statistical significance using Bonferroni correction (shown in bold).

[c] Forced into model.