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Red blood cell membrane α-linolenic acid and the risk of sudden cardiac arrest

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Abstract

Higher levels of long-chain n-3 polyunsaturated fatty acids in red blood cell membranes are associated with lower risk of sudden cardiac arrest. Whether membrane levels of α-linolenic acid, a medium-chain n-3 polyunsaturated fatty acid, show a similar association is unclear. We investigated the association of red blood cell membrane α-linolenic acid with sudden cardiac arrest risk in a population-based case-control study. Cases, aged 25 to 74 years, were out-of-hospital sudden cardiac arrest patients attended by paramedics in Seattle, WA (n = 265). Controls, matched to cases by age, sex, and calendar year, were randomly identified from the community (n = 415). All participants were free of prior clinically diagnosed heart disease. Blood was obtained at the time of cardiac arrest (cases) or at the time of an interview (controls). Higher membrane α-linolenic acid was associated with a higher risk of sudden cardiac arrest: after adjustment for matching factors and smoking, diabetes, hypertension, education, physical activity, weight, height, and total fat intake, the odds ratios corresponding to increasing quartiles of α-linolenic acid were 1.7 (95% confidence interval [CI], 1.0-3.0), 1.9 (95% CI, 1.1-3.3), and 2.5 (95% CI, 1.3-4.8) compared with the lowest quartile. The association was independent of red blood cell levels of long-chain n-3 fatty acids, *trans*-fatty acids, and linoleic acid. Higher membrane levels of α-linolenic acid are associated with higher risk of sudden cardiac arrest.

1. Introduction

Sudden cardiac death, also known as *out-of-hospital sudden cardiac arrest* (SCA), is the leading cause of death from coronary heart disease [1]; and the prevention of SCA in the community remains a challenge [2]. There is strong evidence from epidemiologic studies and clinical trials that dietary intake of long-chain n-3 polyunsaturated fatty acids from seafood reduces the risk of SCA [3]. Among persons without prior clinical coronary disease, both dietary long-chain n-3s and membrane or whole blood levels of these fatty acids are consistently associated with lower risk of SCA [4,5]; and membrane levels are suggested to mediate the dietary association [4].

α-Linolenic acid (ALA) is a medium-chain n-3 polyunsaturated fatty acid (PUFA) and essential fatty acid derived from vegetable oils such as canola and soybean oils. Dietary ALA can be elongated and desaturated to the long-chain n-3 PUFAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in animals, although the conversion appears limited to less than 10% in humans [6]. Dietary intake of ALA is suggested to reduce risk of cardiac death [7,8]. In a small nested case-control study, we found that higher plasma phospholipid ALA levels tended to be associated with lower risk of fatal ischemic heart disease [9]. Whether membrane levels of ALA are also related to SCA risk has received limited attention.

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Using blood specimens collected by paramedics in the field at the time of cardiac arrest and population-based controls, we have shown associations of lower levels of EPA + DHA and higher levels of *trans*-isomers of linoleic acid (*trans*-18:2) in red blood cell (RBC) membranes with higher risk of SCA [4,10]. In this report, we examine the association of red cell membrane ALA and SCA risk after additional data collection.

2. Materials and methods

The study is a continuation of a population-based case-control study, and most of the study subjects were included in earlier reports of PUFAs and SCA risk [4,10].

2.1. Study subjects

Cases were out-of-hospital SCA patients attended by paramedics in Seattle and suburban King County, Washington, between October 1988 and September 2005. We defined *SCA* as a sudden pulseless condition in the absence of evidence of a noncardiac cause of cardiac arrest. Cases were identified from emergency service incident reports. In addition to incident reports, we reviewed death certificates, medical examiner reports, and autopsy reports when available to exclude patients with cardiac arrest due to a noncardiac cause.

We restricted SCA cases to married residents of King County, Washington, between the ages of 25 and 74 years. Cases for whom the paramedics were unable to draw a blood sample at the time of the arrest were excluded. We have previously shown that the distributions of risk factors among cases with and without blood were similar [4]. Because the focus of the study was on persons who appeared healthy until their cardiac arrest, we excluded cases with a history of clinically recognized heart disease or life-threatening comorbidities. We also excluded users of fish oil supplements because fish oil use would affect RBC membrane fatty acid composition.

We were able to contact 89% of the spouses of identified cases. The spouses of 289 eligible cases (82%) participated in an in-person interview (n = 265) or a telephone interview (n = 24), for an overall 73% response rate. In addition, 24 cases were excluded because their blood could not be analyzed because of fatty acid oxidation.

For each case, 1 to 2 control subjects matched on age (within 7 years) and sex were randomly selected from the community by the sampling technique of random-digit dialing [11]. Ninety-four percent of known residential households contacted were successfully screened to determine if residents were eligible for the study. We obtained blood samples and spouse interviews from 415 eligible control subjects (59%), for an overall response rate of 55%. Controls were excluded using the same eligibility criteria as the cases. The University of Washington Human Subject Review Committee approved the study protocol, and all study subjects or their proxy signed an informed consent.

2.2. RBC membrane fatty acid measurements

Paramedics obtained blood specimens from the cases in the field after essential emergency medical care had been provided and either the patient was clinically stable or resuscitation had proven ineffective, usually within 30 to 45 minutes of the cardiac arrest. Blood specimens from controls were obtained at the time of the interview.

Blood specimens were processed [4] and submitted to gas chromatography [12] according to published methods. Laboratory analyses were conducted by technicians blinded to case and control status. Quality control included the use of pooled RBCs and internal standards. Fatty acid levels were expressed as percentages of total fatty acids.

2.3. Relationship of RBC ALA to dietary ALA and other nutrients

We administered a food frequency questionnaire to 81 controls. The questionnaire was developed at the Fred Hutchinson Cancer Research Center, Nutrition Assessment Shared Resource (Seattle, WA) [13]. For each food item, controls were asked to estimate usual serving size and frequency of consumption of 120 line items during the prior month. Nutrient intake was estimated from the questionnaire database that is derived from the University of Minnesota Nutrition Coding Center nutrient database. In this subset of the control population, the average total fat intake was 36.0% of total energy with 7.8% energy from polyunsaturated fat; and mean dietary intake of ALA was 1.9 g/d. The RBC membrane ALA levels were modestly related to the estimate of ALA intake adjusted for total caloric intake (r = 0.21, P = .06). The RBC membrane levels of ALA were not related to total caloric intake (r = 0.04) and to saturated fat intake (r = −0.01).

2.4. Other risk factors assessment

We collected information on demographic factors, medical conditions, lifestyle characteristics, and dietary habits during the spouse interview. Dietary saturated fat intake was assessed with the Northwest Lipid Research Clinic Fat Intake Scale, an index that correlates with saturated fat intake [14]. Dietary intake of long-chain n-3 fatty acids from seafood during the prior month was assessed using the Seafood Intake Scale questionnaire, an instrument that includes a list of 35 types of seafood available in the Pacific Northwest [4]. Mean dietary intake of DHA + EPA from seafood estimated with the Seafood Intake Scale questionnaire in all the study controls was 6.4 g/mo and correlated with RBC membrane levels of DHA + EPA (r = 0.54).

2.5. Statistical analysis

Statistical analyses were carried out using STATA 8.2 (StataCorp, College Station, TX). We compared the distribution of risk factors among cases and controls using

Table 1
Characteristics of cases and controls

Characteristic	Cases, n = 265	Controls, n = 415	*P* value
Age, y, mean (SD)	58.4 (10.5)	57.1 (10.4)	[a]
Women, %	18.5	18.9	[a]
White, %	88.7	92.1	.14
Education, high school graduate, %	71.7	79.5	.02
Current smokers, %	28.9	8.6	<.001
Hypertension, %	24.9	15.2	.002
Diabetes, %	12.6	6.3	.004
Family history of MI or sudden death, %	52.7	43.2	.02
Weight, kg, mean (SD)	85.0 (18.2)	83.2 (15.9)	.19
Physical activity, kcal/wk, mean (SD)	966.8 (1263.0)	1301.8 (1403.8)	.002
Alcohol intake, drinks/d, mean (SD)	1.1 (2.4)	0.8 (1.5)	.14
Caffeine intake, mg/d, mean (SD)	350.2 (476.0)	297.1 (442.7)	.14
Fat index score, mean (SD)	21.2 (3.8)	21.4 (3.7)	.38
RBC fatty acids, % of total fatty acids			
ALA (18:3n3)	0.135 (0.04)	0.128 (0.04)	.01
DHA + EPA	4.22 (1.22)	4.66 (1.33)	<.001
trans-18:2 fatty acids	0.19 (0.07)	0.17 (0.06)	.01
trans-18:1 fatty acids	1.60 (0.53)	1.54 (0.48)	.14
Linoleic acid (18:2n6)	9.26 (1.27)	9.10 (1.11)	.08

MI indicates myocardial infarction.
[a] Matching factor.

2-sample *t* test for continuous variables and Pearson χ^2 test for categorical variables. We compared risk factor distribution across quartiles of ALA levels among controls using analysis of variance. We assessed the associations of ALA with other fatty acids among controls using Pearson correlation coefficients.

We used conditional logistic regression to obtain odds ratios (estimates of relative risks) of SCA associated with increasing levels of RBC membrane ALA. Statistical significance was assessed with the likelihood ratio test. Odds ratios associated with upper quartiles of ALA levels were obtained from models with indicator variables for the quartiles using the lowest quartile as reference. In other analyses, ALA was included as a continuous term; and the odds ratio and 95% confidence intervals (CIs) corresponding to the standard deviation among controls were then calculated from the regression estimates. Quadratic terms were not included because they did not improve the fit of the models. Potential interactions between ALA and subject characteristics were evaluated by testing whether addition of cross products between ALA and a subject characteristic improved the model. Models with cross products were then used to calculate odds ratios associated with ALA for subjects with and without the characteristic.

Information was missing on smoking (2 cases and 6 controls), hypertension (7 cases and 6 controls), education (4 cases and 1 control), diabetes (4 cases), weight (6 cases, 24 controls), height (1 case and 2 controls), and index of fat intake (8 cases, 18 controls). The missing values were imputed using a multiple imputation method [15]. We generated 5 imputed data sets using the MVIS procedure in STATA and combined estimates from the imputed data sets using the MICOMBINE procedure. Results obtained with imputed values are presented in this report. Similar results were obtained when the analyses were restricted to those matched case-control pairs without missing values.

3. Results

The study included 265 cases of SCA without previously diagnosed heart disease and 415 individually matched controls. Given the matching design, mean age and sex distribution were similar in cases and controls (Table 1). As expected, other traditional risk factors for SCA including current smoking, diabetes, hypertension, and family history of myocardial infarction or sudden cardiac death were more prevalent among cases than among controls (Table 1). In addition, cases were less likely to have formal education beyond high school and were less likely to engage in leisure time physical activity.

Mean RBC ALA levels were higher in cases than controls (Table 1). As reported previously in a subset of the current study sample [4,10], mean levels of DHA and EPA were lower and mean levels of *trans*-18:2 were higher in cases (Table 1).

The distribution of covariates across quartiles of RBC membrane levels of ALA among controls is shown in Table 2. α-Linolenic acid levels were not related to age, diabetes, hypertension, smoking, and education (Table 2). However, they were associated with female sex, lower body

Table 2
Distribution of covariates and fatty acids in quartiles of RBC membrane ALA among control subjects

Characteristics	Q1, n = 103	Q2, n = 102	Q3, n = 106	Q4, n = 104	*P* value[a]
Age, y	57.9	57.8	55.4	57.3	.28
Women, %	7.8	19.6	21.7	26.9	.004
White race, %	95.2	90.2	92.5	90.4	.53
Family history of MI, %	45.1	39.6	42.7	45.5	.84
Current smoking, %	9.8	7.0	7.6	9.9	.83
Diabetes, %	8.7	4.9	8.5	2.9	.23
Hypertension, %	15.5	16.7	13.2	15.4	.92
High school education, %	81.6	81.4	75.5	79.6	.68
Weight, kg	88.5	83.6	81.1	79.7	.0006
Physical activity, kcal/wk	1090.7	1326.7	1481.3	1303.5	.25
Alcohol intake, drinks/d	1.04	0.55	0.82	0.94	.11
Caffeine intake, mg/d	268.5	266.3	305.1	347.2	.51
Fat index score (11-29)	22.4	21.8	20.6	21.0	.003
RBC fatty acids[b]					
Linoleic acid (18:2n6)	8.55	9.00	9.28	9.60	<.0001
trans-18:2 fatty acids	0.15	0.17	0.17	0.19	.0001
trans-18:1 fatty acids	1.42	1.57	1.55	1.61	.03
DHA + EPA	4.56	4.57	4.78	4.71	.57

[a] *P* value from analysis of variance.
[b] Percentage of total fatty acids.

weight, and lower fat index score, a dietary measure correlated with both total and saturated fat intake (Table 2). In addition, ALA was positively associated with RBC membrane levels of linoleic acid ($r = 0.39$), levels of *trans*-18:2 ($r = 0.22$), and levels of EPA ($r = 0.16$), but not with levels of DHA ($r = 0.04$).

The RBC membrane levels of ALA were associated with a higher risk of SCA. After adjustment for risk factors, the odds ratios associated with increasing quartiles of ALA were 1.7, 1.9, and 2.5, compared with the lowest quartile (Table 3). The association was unchanged by adjustment for RBC membrane levels of DHA + EPA and *trans*-fatty acids (Table 3), and adjustments for alcohol consumption, caffeine consumption, and RBC membrane levels of linoleic acid (not shown).

An increase in ALA corresponding to 1 standard deviation was associated with 32% higher risk of SCA (odds ratio, 1.32; 95% CI, 1.07-1.63), after adjustment for smoking, diabetes, hypertension, education, kilocalories of leisure time physical activity, fat index, weight, and height. Fig. 1 shows the odds ratios associated with 1 standard deviation of ALA in subgroups defined by age, sex, smoking, diabetes, weight, index of fat intake, dietary DHA + EPA, and RBC membrane levels of DHA + EPA, 18:2n6, and *trans*-fatty acids. Most noticeably, higher ALA level was not associated with lower risk in any subgroup. The association of ALA with SCA risk was not modified by any patient characteristics, except possibly body weight: ALA was associated with higher risk of SCA among subjects who weighed at least 82 kg (the median body weight), but not among subjects with lower weight (P for interaction = .03).

Because the benefits of dietary ALA may be more pronounced in the absence of long-chain n-3 fatty acids [16], we sought to characterize the association of RBC ALA among low seafood eaters. Among 185 subjects with seafood intake less than 1.6 g/mo (the 25th percentile, corresponding to approximately 1 fatty fish meal during a 3-week period), the odds ratio associated with 1 standard deviation of ALA was 1.17 (95% CI, 0.83-1.66) and did not differ significantly from the odds ratio among subjects with higher seafood intake (P for interaction = .30).

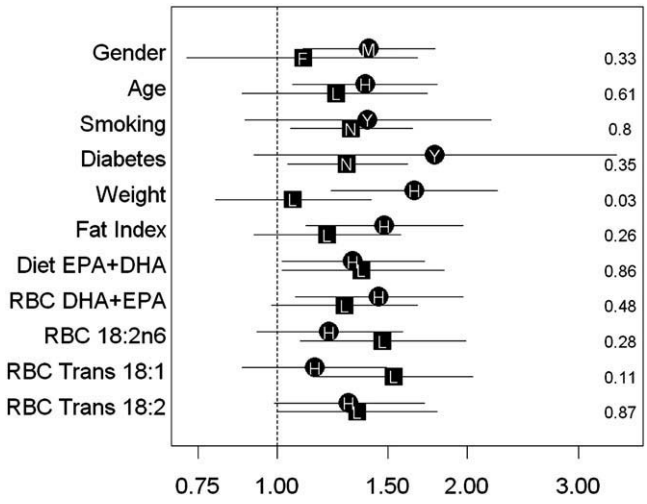

Fig. 1. Association of ALA with SCA according to subject characteristics. The figure shows odds ratios and 95% CIs corresponding to a 1–standard deviation increase in RBC membrane ALA levels for groups with and without several characteristics. The numbers at the right of the figure are P values for test of interactions ("Materials and methods"). For example, the first odds ratio is for men and the second is for women; and the first P value is for the test of interaction with sex. Median values are as follows: age, 57 years; weight, 82 kg; fat index, 21; dietary DHA + EPA, 4.0 g/mo; RBC DHA + EPA, 4.53% of total fatty acids; RBC 18:2n6, 9.03% of total fatty acids; RBC *trans*-18:1 fatty acids, 1.48% of total fatty acids; and RBC *trans*-18:2 fatty acids, 0.173% of total fatty acids. M indicates men; W, women; Y, yes, N, no; H, *high* (defined as greater than or equal to the median value); L, *low* (defined as less than the median value).

Table 3
Association of RBC membrane ALA with SCA

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P for trend
Cases/ controls, n	45/103	68/102	70/106	82/104	
Odds ratio (95% CI)					
Model 1	1.0 (Ref)	1.59 (0.96-2.62)	1.62 (1.00-2.62)	1.98 (1.13-3.47)	.02
Model 2	1.0 (Ref)	1.70 (0.97-2.99)	1.91 (1.11-3.28)	2.54 (1.33-4.83)	.005
Model 3	1.0 (Ref)	1.51 (0.85-2.70)	1.80 (1.04-3.13)	2.43 (1.26-4.68)	.007
Model 4	1.0 (Ref)	1.48 (0.82-2.66)	1.71 (0.97-2.99)	2.22 (1.14-4.31)	.02

Model 1: adjusted for matching factors and continuous age only; model 2: as model 1, further adjusted for smoking, diabetes, hypertension, education, kilocalories of leisure time physical activity, fat index, weight, and height; model 3: as model 2, further adjusted for levels of RBC DHA + EPA; model 4: as model 2, further adjusted for levels of RBC DHA + EPA and *trans*-18:2 fatty acids.

4. Discussion

In this population-based study, higher levels of ALA in RBC membranes were not associated with a reduction in risk of SCA. On the contrary, we observed an association with higher risk. The association was independent of traditional risk factors and membrane levels of other fatty acids and consistent across a variety of subgroups, including subjects with lower intake of long-chain n-3 PUFAs.

The study results contrast with suggested benefits of dietary ALA. Several cohort studies have investigated the relation of ALA in the diet and sudden cardiac death or fatal heart disease. In the Nurses Health Study, higher intake of ALA was associated with lower risk of sudden cardiac death [7]. The benefits were less clear among the men in the Health Professionals cohort, where dietary ALA was not associated with risk of sudden cardiac death (hazard ratio for each 1 g/d, 1.2; 95% CI, 0.7-1.9) [16]. In cohort studies among men at high risk of heart disease, high levels of dietary ALA were

associated with a nonsignificant lower risk of fatal heart disease [17,18]. A meta-analysis of cohort findings reported a relative risk of fatal heart disease of 0.8 (0.6-1.0) associated with an increase in ALA of 1.2 g/d [19]. There is limited information on dietary ALA and coronary heart disease events from clinical trials. In the Lyon Diet Heart Study, consumption of a Mediterranean diet, enriched in ALA, decreased the risk of cardiac death in patients with a prior myocardial infarction [20]. However, the experimental diet involved many dietary changes; and it is not certain that the higher ALA intake contributed to the reduced risk of cardiac death. In summary, dietary studies suggest a possible lower risk of sudden cardiac death and fatal heart disease with dietary ALA.

Although dietary ALA might be associated with lower risk, the present study suggests that RBC membrane ALA, by contrast, is associated with higher risk of SCA. The correlation between diet and RBC levels of ALA was modest ($r = 0.21$ among study controls), similar to that reported in other studies [21]. Measurement error would be expected to lower a true correlation between dietary intake and membrane ALA levels. However, other factors, such as metabolic processes under genetic control, also may influence the relation between dietary intake and cell membrane levels and result in variation in cell membrane ALA. In support of this possibility, we have shown heritability of most membrane fatty acids [22]. Because factors other than diet might influence membrane ALA levels, it is possible that the associations of dietary ALA and membrane ALA with SCA risk are discordant.

Experimental animal studies do not suggest a higher risk of arrhythmia with higher plasma levels of free ALA [23], and ALA does not appear to be proarrhythmic in cell experiments [24]. However, higher levels of membrane ALA may reflect a metabolic process itself associated with risk. It is noteworthy that only a fraction of dietary ALA, less than 10% in tracer studies of healthy volunteers [6], is incorporated in the plasma phospholipid pool. However, most of the ALA that is incorporated in plasma phospholipids is converted to EPA [6]. Short-term dietary trials with large doses of ALA result in higher plasma levels of EPA that may mediate observed benefits on risk factors [25]. It is also possible that conversion to EPA mediates the association of dietary ALA with lower risk of SCA. We hypothesize that high levels of RBC membrane ALA are a marker of poor conversion to EPA, perhaps explaining the association with higher SCA risk. For example, genetic variation in Δ6-desaturase, a key enzyme in the conversion from ALA to EPA, is associated with lower levels of EPA in adipose tissue [26]. Future studies will be needed to investigate if the association of dietary ALA with SCA is modified by gene variation.

We considered the possibility that the benefit of ALA may be affected by intake of long-chain n-3 PUFAs. However, we observed similar associations of RBC membrane ALA with higher risk of SCA among subjects with low and high intake of DHA + EPA and among subjects with low and high RBC membrane levels of DHA + EPA. In the Health Professionals cohort, estimates of the risk of sudden death associated with high dietary intake of ALA among men suggested a lower risk only in the context of low DHA + EPA intake; but the CIs of the estimates were very large and overlapping [16]. In contrast, dietary ALA was associated with lower risk of sudden cardiac death in women with high and low intake of DHA + EPA in the Nurses Health Study [7].

The possibility that the association of ALA with higher risk of SCA was restricted to subjects with higher body weight is intriguing, although this observation may have been due to chance given the number of subgroups examined. In a dietary trial among men and women with the metabolic syndrome and an average body mass index of 35, high dietary consumption of walnuts, rich in ALA and total PUFA, unexpectedly lowered the baroreflex sensitivity [27]. A depressed baroreflex sensitivity is a marker of impaired autonomic control and a risk factor for arrhythmias [28]. Further studies are needed to confirm that ALA increases the risk of SCA among overweight subjects.

Cell membrane levels of ALA were correlated with levels of linoleic acid, the major PUFA in the diet; however, linoleic acid did not account for the association of ALA with an increase in SCA risk. In addition, ALA was associated with *trans*-18:2; and *trans*-18:2 are associated with higher risk of SCA [10,29]. We considered the possibility that ALA might be a marker of *trans*-18:2 but rejected the possibility on the basis of the following arguments. Adjustment of the analysis of ALA with SCA for levels of *trans*-18:2 reduced the odds ratio associated with 1 standard deviation of ALA by 10%, from 1.32 to 1.29. For *trans*-18:2 to account for all the association of ALA with SCA, we would have to assume a measurement error in *trans*-18:2 on the order of 90%. With such large measurement error in *trans*-18:2, we would not have been able to detect an association of *trans*-18:2 with risk; or the true association would be unreasonably large. Furthermore, with such large error, we would not detect the observed correlation of 0.21 with ALA.

The strengths of this study include the use of population-based cases and controls, the objective assessment of ALA in RBC membranes, and the adjustment of results for other known risk factors. To address the possibility that cases might have changed their diet as a consequence of poor health leading to SCA, we restricted the study to cases with no history of clinically recognized heart disease and no life-threatening comorbidities.

Several limitations are noteworthy. We only had a measure of dietary ALA in a small subset of controls, and we could not contrast the associations of diet and membrane levels ALA with SCA within this study population. Because of incomplete dietary assessment, the possibility of residual confounding cannot be eliminated. In particular, we may have incompletely adjusted for saturated fat intake. However, we did not find evidence of a relation

of RBC membrane ALA with saturated fat intake among control subjects with a more comprehensive dietary assessment. It is therefore unlikely that differences in saturated fat intake account for the observed association between ALA and SCA. The use of surrogate respondents inevitably introduced some misclassification in assessment of potential confounders; however, the exposure of interest was measured objectively. Participation rate in the controls was 60%, and the odds ratios associated with higher levels of ALA could be overestimated if controls who declined participation in the study ate more ALA-containing foods than the controls who participated. Despite this limitation, our previously reported findings in this study population on dietary intake and cell membrane levels of n-3 PUFA and the risk of SCA have been replicated in prospective cohort studies [5,30,31].

In conclusion, we observed an association of RBC membrane levels of ALA with higher risk of SCA. We hypothesize that membrane ALA is a marker of poor conversion to long-chain n-3 PUFAs. Further work is needed to confirm the study findings in other populations and to explore whether the association of dietary ALA with SCA is affected by variation in metabolic processes, such as incorporation into membrane phospholipids and conversion to EPA.

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