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Red Blood Cell Membrane Concentration of Cis-Palmitoleic and Cis-Vaccenic Acids and Risk of Coronary Heart Disease

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Abstract

Although previous studies have suggested associations between plasma palmitoleic acid and coronary heart disease (CHD) risk factors including blood pressure, inflammation, and insulin resistance, little is known about the relation of palmitoleic acid with CHD. This ancillary study of the Physicians' Health Study was designed to examine whether red blood cell (RBC) membrane *cis*-palmitoleic acid and *cis*-vaccenic acid – two fatty acids that can be synthesized endogenously – are associated with CHD risk. We used a risk set sampling method to prospectively select 1000 incident CHD events and 1000 matched controls. RBC membrane fatty acids were measured using gas chromatography. CHD cases were ascertained using annual follow-up questionnaire and validated by an Endpoint Committee through review of medical records. In a conditional logistic regression adjusting for demographics, anthropometric, lifestyle factors, and comorbidity, odds ratios (95% CI) for CHD were 1.0 (ref), 1.29 (0.95–1.75), 1.08 (0.78–1.51), 1.25 (0.90–1.75), and 1.48 (1.03–2.14) across consecutive quintiles of RBC membrane *cis*-palmitoleic acid (p for trend 0.041). Odds ratio associated with each standard deviation higher RBC membrane *cis*-palmitoleic acid was 1.19 (95% CI: 1.06–1.35) in a multivariable adjusted model. Lastly, RBC membrane *cis*-vaccenic acid was inversely associated with CHD risk [OR: 0.79 (95% CI: 0.69–0.91) per standard deviation increase]. In conclusion, our data showed a positive association between RBC membrane *cis*-palmitoleic acid and CHD risk in male physicians. Furthermore, RBC membrane *cis*-vaccenic acid was inversely related to CHD.

Keywords

Coronary artery disease; epidemiology; fatty acids; *cis* palmitoleic acid

Despite a decline in mortality from coronary heart disease (CHD) from its peak in the late 1960s, CHD still carries a disproportionately high burden in the US^{1,2}. Data in the literature

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on the role of various fatty acids on the risk of developing CHD have been inconsistent or limited, especially for fatty acids that can be synthesized endogenously via de novo lipogenesis (DNL) pathway (also referred to as fatty acid synthesis). DNL reflects the body's adaptation to handle high-carbohydrate loads through conversion of excess carbohydrate to fatty acids and triglycerides^{3,4}. DNL pathway involves endogenous utilization of acetyl-coA to produce saturated fatty acids (i.e. 16:0 and 18:0), which can then be elongated and desaturated to generate other fatty acids such as palmitoleic acid (16:1n-7), oleic acid (18:1 n-9), or vaccenic acid (18:1-n7)⁵. Heightened hepatic DNL may lead to hypertriglyceridemia³ with subsequent progression of atherosclerosis and development of CHD⁶. While emerging data suggest that these fatty acids generated from DNL may influence the risk of sudden death^{7,8} or CHD risk factors including diabetes⁹, hypertension¹⁰, and inflammation¹¹, only limited and inconsistent data are available on the influence of mono-unsaturated fatty acids 16:1n-7 and 18:1n-7 on the risk of CHD^{7,12} or CHD mortality¹³. Wang et al.¹² reported no association between plasma phospholipid or cholesterol ester fraction of palmitoleic acid and incident CHD. Data from the ARIC study found a positive association between plasma palmitoleic acid and incident diabetes¹⁴. Since diabetes is associated with a two-to four-fold increased risk of CHD¹⁵, these data suggest that palmitoleic acid could influence the risk of CHD. The current ancillary study sought to examine associations between RBC membrane *cis*-palmitoleic acid and *cis*-vaccenic acid and CHD risk among US male physicians.

Methods

Participants in these analyses are members of the Physicians Health Study (PHS) I and II who provided blood samples between 1995 and 2001. The PHS I is a completed randomized trial designed to study the effects of low-dose aspirin and beta-carotene on cardiovascular disease and cancer¹⁶. The PHS II is a randomized trial (started in 1997) designed to study the effects of various vitamins on the risk of cardiovascular disease and cancer¹⁷.

In this ancillary study, we used a prospective nested case-control design with a density sampling technique to randomly select 1,000 pairs of incident CHD cases and matching controls among PHS participants who provided blood samples between 1995 and 2001. Briefly, for each CHD case, we randomly selected one control among participants that were alive and free of confirmed CHD at the time of the index case diagnosis and matched on age at blood collection (within 1 year), year of birth (within 2 years), and time of blood collection (within 3 months). Each case was eligible to serve as a control before CHD diagnosis. Likewise, each control was eligible to later become a CHD case to assure that the control series is a representative sample of the study base that generated all the cases¹⁸. Each study participant gave written informed consent, and the Brigham and Women's Hospital Institutional Review Board approved the study protocol.

We used stored red blood cells collected between 1995 and 2001 for fatty acids assays. All investigators including laboratory personnel were blinded to the case status of all participants. RBC fatty acid profiles were quantified using an established gas chromatography method as previously described¹⁹. Briefly, lipids were extracted from RBC membranes²⁰ followed by saponification and methylation²¹. The resultant fatty acid methyl esters were analyzed using an Autosystem XL gas chromatograph (Perkin Elmer, Boston MA) equipped with a 100m × 0.25mm i.d (film thickness 0.25µm) capillary column (SP-2560, Supelco). Peaks of interest were identified by comparison with authentic fatty acid standards (Nu-Chek Prep, Inc. Elysian, MN) and expressed as molar percentage (mol %) proportions of total fatty acids. Interassay coefficients of variation were <4.5% for fatty acids present at levels >1 mol% and <7.1% for fatty acids present at levels <1 mol%.

Detailed description of cardiovascular events in the PHS has been published^{16,17,22,23}. Briefly, we used annual follow-up questionnaires to initially obtain information on incident CHD, defined in this study as non-fatal or fatal myocardial infarction, percutaneous transluminal coronary angioplasty or coronary artery bypass graft. All CHD events were adjudicated by an endpoint committee based on review of medical records.

Demographic information was obtained through self-reports. At baseline, each subject provided information on exercise, smoking (never, former, and current smoker), and alcohol intake. Self-reported baseline weight and height were used to compute body mass index (weight in kilograms divided by height in meter squared). Information on comorbidity (hypertension, diabetes, hypercholesterolemia, heart failure, atrial fibrillation, left ventricular hypertrophy, etc) was collected at baseline and through follow-up questionnaires. A validated food frequency questionnaire²⁴ was used to collect data on macro- and micronutrients around the time of blood collection (1997–2001).

We used the distribution of RBC membrane *cis*-palmitoleic and *cis*-vaccenic acids in the control series (n=1,000) to create the respective quintiles of *cis*-palmitoleic and *cis*-vaccenic acids. Means and percentages of baseline characteristics of the study participants are presented according to these quintiles. We used conditional logistic regression to estimate the relative risk of CHD using the lowest quintile of each main exposure as the reference category. The initial model adjusted for matching variables (race, age, time of blood collection, and year of birth); a final model also controlled for body mass index; prevalent hypertension; hypercholesterolemia; atrial fibrillation; diabetes; left ventricular hypertrophy; smoking (never, former, current smokers); alcohol intake (<1, 1–6, and 7+ drinks/week); physical activity (rarely/never, 1–3 times per month, 1–4 times per week, and 5+ times per week); and red blood cell membrane 18:0, 16:0, and marine omega-3 fatty acids (20:5n-3, 22:5n-3, and 22:6n-3). To obtain a p value for linear trend, we used a new continuous variable that was assigned median values of *cis*-palmitoleic acid or *cis*-vaccenic acid in the regression model. We repeated above analyses using *cis*-palmitoleic or *cis*-vaccenic acid as a continuous variable and estimated relative risk of CHD associated with one standard deviation increase of log-transformed *cis*-palmitoleic or *cis*-vaccenic acid.

In secondary analyses, we evaluated the association between stearoyl-coA desaturase activity, estimated by calculating the ratio of product –to-substrate (16:1n-7/16:0 and 18:1n-/18:0) as well as elongase activity (18:1n-7/16:1n-7 ratio) and the risk of CHD, using each standard deviation increase in each exposure. Because excess carbohydrate and protein intake can increase hepatic DNL, we conducted a sensitivity analysis by repeating the main analysis with additional adjustment for energy intake, whole grain, energy from carbohydrate and protein (of note is that about 326 participants had missing data on macronutrients). All analyses were performed using SAS (SAS version 9.3, NC) and the alpha level was set at 0.05. All p values were 2-sided.

Results

The mean age (SD) was 68.7 (8.7) years (range 50.4 to 92.0) among 2000 study participants. In the control series, median (IQR) red blood cell concentration of *cis*-palmitoleic and *cis*-vaccenic acids were 0.49 % (0.37%-0.65%) and 1.69% (1.47%-2.00%) of total RBC membrane fatty acids, respectively. Compared to individuals in the lowest quintile, those with higher quintile of *cis*-palmitoleic acid had a higher BMI; energy intake; stearoyl-coA desaturase activity; oleic, palmitic, and *cis*-vaccenic acids; prevalence of hypertension, atrial fibrillation, and hypercholesterolemia; and lower concentration of RBC marine omega-3 fatty acids, and elongase activity, and were less likely to exercise (Table 1). Higher RBC *cis*-vaccenic acid was associated with a lower prevalence of diabetes and atrial fibrillation

(Table 1). In a conditional logistic regression model adjusting for matching factors, vaccenic acid, body mass index, prevalent hypertension, hypercholesterolemia, atrial fibrillation, diabetes, left ventricular hypertrophy, RBC 16:0, 18:0, 20:5n-3, 22:5n-3, and 22:6n-3, smoking, alcohol intake, and exercise, odds ratios (95% CI) for CHD were 1.0 (ref), 1.29 (0.95–1.75), 1.08 (0.78–1.51), 1.25 (0.90–1.75), and 1.48 (1.03–2.14) across consecutive quintiles of RBC *cis*-palmitoleic acid (*p* trend 0.041, Table 2). Each standard deviation increase of *cis*-palmitoleic acid was associated with a 19% higher risk of CHD (95% CI: 6% to 35%) in the fully adjusted model, Table 2. Additional adjustment for whole grain intake, energy, and energy from carbohydrate and protein did not alter the association [OR: 1.24 (95% CI: 1.07–1.44) per standard deviation increase in RBC *cis*-palmitoleic acid].

RBC *cis*-vaccenic acid was inversely associated with the risk of CHD in a stepwise fashion (*p* for linear trend 0.007, Table 3). Each standard deviation increase in *cis*-vaccenic acid was associated with a 21% lower odds of CHD (95% CI: 9% to 31%), in a fully adjusted model, Table 3. Additional adjustment for energy, carbohydrate, protein, and whole grain did not alter the relation [OR=0.82 (95% CI: 0.69–0.98)]. In secondary analyses, we examined the relation of stearoyl-coA desaturase and elongase activities (estimated using product-to-substrate ratio) and observed a positive association of 16:1n-7/16:0 ratio with CHD. Each standard deviation of stearoyl Co-A desaturase activity was associated with a 13% higher risk of CHD (95% CI: 2% to 26%) in a fully adjusted model, Table 4. The relation of 18:1n-9/18:0 ratio with CHD risk did not reach statistical significance in the full model (Table 4). Lastly, there was an inverse association between elongase activity (18:1n-7/16:1n-7 ratio) and CHD risk [OR=0.81 (95% CI: 0.73–0.91) per standard deviation increase, Table 4].

Discussion

In this prospective nested case-control ancillary study of US male physicians, we observed a positive association between RBC *cis*-palmitoleic acid as well as 16:1n-7/16:0 ratio with CHD risk. Furthermore, RBC *cis*-vaccenic acid and elongase activity were inversely associated with CHD risk. These findings were robust after further adjustment of alcohol consumption and energy from carbohydrate and protein – factors that stimulate hepatic DNL.

Limited data are available on the association between *cis*-palmitoleic acid and CHD risk. In a nested-case-control study, total plasma 16:1n-7 (*cis* and *trans* isoforms) was not associated with CHD in men from the Multiple Risk Factor Intervention Trial men [OR: 1.08 (95% CI: 0.77–1.57) per standard deviation of phospholipid fraction and OR: 1.35 (95% CI: 0.96–1.89) for cholesterol fraction]²⁵. Among US older adults from the Cardiovascular Health Study (CHS), plasma phospholipid *cis*-palmitoleic acid was not associated with incident CHD [RR: 0.89 (95% CI: 0.58–1.37) per interquintile range of 16:1n-7]⁷. Furthermore, plasma phospholipid *cis*-vaccenic acid was associated with a non-statistically significant lower risk of total CHD [RR: 0.75 (95% CI: 0.43–1.32) per interquintile range of 18:1n-7]⁷. Unfortunately, the CHS data did not evaluate the relation of stearoyl-coA desaturase or elongase activity on CHD risk for comparison with our findings. The apparent discrepancy between a positive association observed in our study and null effect of palmitoleic acid on CHD risk by others^{7,25} merits some comments.

A difference in age across studies may partially explain the result inconsistency. PHS participants were older than people in the MRFIT²⁵ study (69 y vs. 50 y on average), but younger than people in the CHS (69 vs. 74 y). Furthermore, while PHS measured fatty acids on RBC membranes, MRFIT²⁵ and CHS⁷ utilized plasma phospholipid or cholesterol ester fractions. Although RBC membrane fraction of fatty acids may better reflect long-term

concentration of fatty acids than plasma concentration, it is also influenced by ongoing fatty acid metabolism and exchange between membranes and plasma. Lastly, both MRFIT and CHS estimates of effects have wide confidence intervals that do not exclude the possibility of modest positive association of palmitoleic with CHD as observed in the PHS. Of particular note is the consistency of the estimates of association between CHS and PHS for vaccenic acid (RR: 0.75 in CHS vs. 0.79 in PHS) when vaccenic acid was analyzed as continuous variable; despite a wider 95% CI in the CHS, these point estimates are both suggestive of a lower risk of CHD despite the lack of statistical significance in the CHS data.

Our findings of stearoyl-coA desaturase activity and elongase activity on CHD risk are novel and consistent with the main analyses. If confirmed in other cohorts, these findings may provide an additional pharmacological target to influence DNL. Specifically, inhibition of stearoyl-coA desaturase may reduce the production of 16:1n-7 from palmitic acid (16:0) and thereby avert adverse effects of 16:1n-7. Likewise, pharmacological stimulation of elongase activity may also reduce the concentration of 16:1n-7 via its elongation to synthesize a more favorable vaccenic acid (18:1n-7) and thereby provide novel potential pharmacological targets to reduce the risk of CHD.

Underlying mechanisms by which *cis*-palmitoleic and *cis*-vaccenic acids might influence the risk of CHD have not been elucidated. It is possible that *cis*-palmitoleic acid might increase the risk of CHD via increase in blood pressure. Zheng et al.¹⁰ reported an increased prevalent hypertension per interquartile increment of cholesterol ester 16:1n-7 [OR=1.31 (95% CI: 1.18–1.44)] as well as increased incident hypertension [RR=1.26 (95% CI: 1.11–1.42)]. Other potential mechanisms include pro-inflammatory^{11,26} effects of *cis*-palmitoleic acid. In animal models, palmitoleic acid down-regulated mRNA expression of pro-inflammatory genes (tumor necrosis alpha and resistin) in white adipose tissue and lipogenic genes in the liver²⁶. Lastly, palmitoleic acid may modulate the risk of CHD via insulin/glucose metabolism pathway. Data from the ARIC showed an increased risk of diabetes with plasma palmitoleic acids¹⁴. However, a positive relation of palmitoleic acid with incident diabetes was not confirmed in the US older adults²⁷ and other studies reported favorable effects of palmitoleic acid on diabetes risk^{28,29}. The latter findings are consistent with experimental data, where administration of palmitoleic acid in mice was associated with enhanced whole-body glucose disposal and improved insulin sensitivity²⁶. Overall, there is a need to further elucidate underlying biologic mechanisms by which palmitoleic acid may influence the risk of CHD in humans.

Our study has some limitations. The fact that all participants were male physicians, most of whom were Caucasians, limits the generalizability of the current findings to other ethnic groups, females, and people with lower educational attainment. Having only one measurement of RBC membrane fatty acids did not allow us to account for temporal changes in RBC membrane fatty acid concentrations. Given the observational nature of the study design, we cannot rule out chance, residual or unmeasured confounding as alternative explanation of our results. On the other hand, the use of matching to minimize confounding, the availability of several potential covariates, use of reproducible biomarkers to assess fatty acids, and standardized methods for follow-up and cases ascertainment in PHS are strengths of the present study.

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Table 1

Characteristics of 2000 male physicians by quintiles of red blood cell membrane *cis*-palmitoleic and *cis*-vaccenic acids *

	<i>Cis</i> -palmitoleic acid (16:1n-7)				<i>Cis</i> -vaccenic acid (18:1n-7)			
	Q1 (low)	Q3	Q5 (high)		Q1 (low)	Q3	Q5 (high)	
Mean (SD)	0.28 (0.05)	0.49 (0.02)	0.93 (0.23)		1.28 (0.11)	1.69 (0.06)	2.52 (0.47)	
Characteristics	(N=373)	(N=377)	(N=440)		(N=433)	(N=407)	(N=393)	
Age (years)	69.4±8.4	68.4±8.7	67.6±8.4		68.0±8.1	69.5±8.6	68.1±9.5	
Body mass index (kg/m ²)	25.3±3.2	26.0±3.3	26.9±3.9		26.2±3.3	25.9±3.4	26.2±3.9	
<i>cis</i> vaccenic acid †	1.59±0.29	1.76±0.43	2.01±0.66		1.28±0.11	1.69±0.06	2.52±0.47	
16:1n-7/16:0 ratio	0.013±0.002	0.022±0.002	0.038±0.009		0.021±0.009	0.023±0.009	0.029±0.011	
18:1n-9/18:0 ratio	0.766±0.124	0.835±0.134	0.941±0.190		0.817±0.178	0.867±0.163	0.871±0.142	
18:1n-7/16:1n-7 ratio	5.83±2.33	3.58±0.89	2.26±0.83		3.11±1.52	3.67±1.43	4.19±1.64	
Oleic acid <i>cis</i> (18:1n-9)	13.4±1.2	14.2±1.4	15.4±2.0		13.9±1.5	14.4±1.79	14.8±1.8	
Palmitic acid (16:0) †	21.8±1.7	22.9±2.2	24.6±2.9		22.6±1.9	23.3±2.6	23.7±2.9	
Stearic acid (18:0) †	17.9±3.2	17.3±2.9	16.9±3.5		17.8±4.4	17.0±2.5	17.2±2.0	
Marine omega-3 †	6.63±1.91	6.06±1.82	5.35±1.85		6.43±1.75	6.12±2.02	5.37±1.93	
Energy intake (kcal) †	1672±534	1685±547	1680±488		1688±498	1682±524	1666±507	
Energy from carbohydrate (%) †	51.8±9.7	50.6±9.7	49.3±10.4		49.7±9.8	50.0±9.2	50.9±10.9	
Energy from protein (%) †	18.7±3.4	18.3±3.3	17.9±3.3		18.4±3.3	18.5±3.1	18.3±3.6	
Whole grain (servings/week) †	4.71±1.88	4.43±1.85	4.56±1.70		4.48±1.77	4.47±1.94	4.53±1.79	
Diabetes mellitus	10.2%	8.0%	8.2%		9.7%	9.8%	5.9%	
Hypertension †	53.0%	52.7%	59.1%		53.7%	57.6%	49.6%	
Atrial fibrillation	10.5%	8.5%	10.7%		10.4%	7.4%	10.2%	
Hypercholesterolemia †	29.5%	30.2%	39.3%		32.3%	32.2%	29.0%	
Heart failure	1.9%	2.4%	2.5%		0.7%	2.7%	3.3%	
LV hypertrophy	0.8%	3.5%	2.1%		1.9%	3.0%	2.8%	
Current smoking	0.8%	1.9%	5.7%		2.1%	4.9%	3.3%	
Current drinkers	71.3%	81.4%	87.0%		80.6%	79.4%	80.7%	
Current exercise †	65.0%	61.9%	60.5%		62.6%	62.2%	66.1%	

* We used quintile distribution of fatty acids in the control series. Data are presented as means $\pm$ standard deviation or percentages.

[†] Fatty acids are expressed as molar percentage of total red blood cell membrane fatty acids.

[‡] There were missing data on energy, carbohydrate, or protein intake (n=326); whole grain intake (n=246) and exercise (n=22); hypertension defined as systolic blood pressure $\geq$ 140 or diastolic blood pressure $\geq$ 90 mm Hg or use of antihypertensive drugs; hypercholesterolemia defined as total cholesterol $\geq$ 200 mg/dl or use of cholesterol lowering drugs.

Odds ratios for coronary heart disease by quintiles or per standard deviation increase of red blood cell membrane *cis*-palmitoleic fatty acids in the Physicians' Health Study

Table 2

Odds ratio (95% CI) for coronary heart disease				
Quintiles [Range] of <i>cis</i> Palmitoleic acid (16:1n-7)	Cases	Model 1 [*]	Model 2 [†]	
Q1 (low) [0.04–0.38] [‡]	173	1.0	1.0	
Q2 [0.39–0.48]	205	1.23 (0.92–1.64)	1.29 (0.95–1.75)	
Q3 [0.49–0.59]	177	1.10 (0.82–1.49)	1.08 (0.78–1.51)	
Q4 [0.60–0.90]	205	1.29 (0.95–1.74)	1.25 (0.90–1.75)	
Q5 (high) [>0.91]	240	1.58 (1.16–2.15)	1.48 (1.03–2.14)	
P for trend		0.003	0.04	
Per SD increase of log-palmitoleic acid (<i>cis</i> 16:1n-7)		1.20 (1.09–1.32)	1.19 (1.06–1.35)	

^{*} Model 1 adjusted for matching variables and *cis*-vaccenic acid; SD=standard deviation of log-transformed red blood cell membrane *cis*-palmitoleic acid

[†] Model 2 adjusted for matching variables plus body mass index, prevalent hypertension, hypercholesterolemia, atrial fibrillation, diabetes, left ventricular hypertrophy, smoking, alcohol intake, physical activity, and red blood cell membrane 18:0, 16:0, and marine omega-3 fatty acids (20:5n-3, 22:5n-3, and 22:6n-3)

[‡] Fatty acids expressed as percentage of total red blood cell membrane fatty acids

Odds ratios for coronary heart disease by quintiles or per standard deviation increase of red blood cell membrane *cis*-vaccenic fatty acids in the Physicians' Health Study

Table 3

Odds ratio (95% CI) for coronary heart disease				
Quintiles [Range] of <i>cis</i> -vaccenic acid (18:1n-7)	Cases	Model 1*	Model 2†	
Q1 (low) [0.90–1.49]‡	233	1.0	1.0	
Q2 [1.50–1.68]	191	0.76 (0.56–1.02)	0.75 (0.55–1.03)	
Q3 [1.69–1.92]	208	0.74 (0.54–1.03)	0.66 (0.47–0.94)	
Q4 [1.93–2.53]	175	0.60 (0.42–0.85)	0.55 (0.38–0.81)	
Q5 (high) [>2.54]	193	0.59 (0.40–0.88)	0.56 (0.37–0.85)	
P for trend		0.01	0.007	
Per SD increase of log-vaccenic acid (<i>cis</i> 18:1n-7)		0.80 (0.70–0.91)	0.79 (0.69–0.91)	

* Model 1 adjusted for matching variables and *cis*-palmitoleic acid; SD=standard deviation of log-transformed red blood cell membrane *cis*-palmitoleic acid

† Model 2 adjusted for matching variables plus body mass index, prevalent hypertension, hypercholesterolemia, atrial fibrillation, diabetes, left ventricular hypertrophy, smoking, alcohol intake, physical activity, and red blood cell membrane 18:0, 16:0, and marine omega-3 fatty acids (20:5n-3, 22:5n-3, and 22:6n-3)

‡ Fatty acids expressed as percentage of total red blood cell membrane fatty acids

Odds ratios for coronary heart disease by per standard deviation increase of log-transformed stearoyl-coA desaturases and elongase activity in the Physicians' Health Study^{*}

Table 4

Per SD of log-transformed	Odds ratio (95% CI) for coronary heart disease	
	Model 1 [†]	Model 2 [‡]
Stearoyl-coA desaturase (16:1n-7/16:0 ratio)	1.15 (1.04–1.27)	1.13 (1.02–1.26)
Stearoyl-coA desaturase (18:1n-9/18:0 ratio)	1.13 (1.00–1.28)	1.08 (0.86–1.36)
Elongase activity (18:1n-7/16:1n-7 ratio)	0.82 (0.75–0.90)	0.81 (0.73–0.91)

^{*} Desaturase and elongase activities were estimated using the ratio of product-to-substrate.

[†] Model 1 adjusted for matching variables; SD=standard deviation of log-transformed red blood cell membrane *cis* palmitoleic acid

[‡] Model 2 adjusted for matching variables plus body mass index, prevalent hypertension, hypercholesterolemia, atrial fibrillation, diabetes, left ventricular hypertrophy, smoking, alcohol intake, physical activity, and red blood cell membrane 18:0, 16:0, and marine omega-3 fatty acids (20:5n-3, 22:5n-3, and 22:6n-3)