

Association Between Fatty Acids of Blood Cell Membranes and Incidence of Coronary Heart Disease

A Case-Control Study Nested in the PREDIMED Trial

Christopher Papandreou, Aleix Sala-Vila, Serena Galié, Jananee Muralidharan, Ramón Estruch, Montserrat Fitó, Cristina Razquin, Dolores Corella, Emilio Ros, Juan Timiraos, Jose Lapetra, Lluís Serra-Majem, Silvia Carlos, Olga Castañer, Eva M. Asensio, Jordi Salas-Salvadó, Mònica Bulló

Objective—To examine the associations between baseline levels of fatty acids in blood cell membranes and their 1-year changes with the incidence of coronary heart disease (CHD) in older adults at high cardiovascular disease risk.

Approach and Results—This is a case-control study nested in the PREDIMED trial (Prevenció con Dieta Mediterránea), with 136 CHD cases and 272 controls (matched on age, sex, body mass index, intervention group, and time of permanence in the study to the time event). We used gas chromatography to measure the proportion of 22 fatty acids in blood cell membranes at baseline and after 1 year. Conditional logistic regression was used to estimate odds ratios (ORs) and 95% CIs. After adjustment for classical CHD risk factors and multiple testing, 1 SD increase in baseline levels of C22:0, C24:0 and the sum of individual very long chain saturated fatty acids was associated with 56% (OR, 0.44 [95% CI, 0.28–0.69]), 59% (OR, 0.41 [95% CI, 0.25–0.65]), and 55% (OR, 0.45 [95% CI, 0.29–0.70]) a decreased odds of developing CHD, respectively. Baseline C20:1n9 was associated with higher odds of CHD (OR, 1.58 [95% CI, 1.25–2.00]).

Conclusions—Higher levels of C22:0 and C24:0 were associated with a lower CHD incidence, whereas higher levels of C20:1n9 were associated with a higher risk. This study adds to the growing body of evidence suggesting potential differences in the cardiovascular disease effects of different types of circulating saturated fatty acids.

Visual Overview—An online [visual overview](#) is available for this article. (*Arterioscler Thromb Vasc Biol.* 2019;39:819–825. DOI: 10.1161/ATVBAHA.118.312073.)

Key Words: body mass index ■ cell membrane ■ fatty acids ■ heart diseases ■ incidence

Coronary heart disease (CHD), also known as ischemic heart disease, is the leading cause of death worldwide.¹ CHD incidence is reduced by the appropriate management of risk factors.² A meta-analysis summarizing 14 relevant clinical trials showed that 15.4% of women and 19.4% of men with CHD had no classical risk factors.³ This reinforces the need of identifying novel risk markers for CHD to improve the selection of individuals for preventative strategies.

Fatty acids are integral compounds of cell membrane phospholipids. Rather than merely acting as inert structural elements, fatty acids modulate the physicochemical properties

of the membrane and can be converted to bioactive molecules once released from the membrane, having a significant impact on health and disease.⁴

In relation to CHD, there is a large body of evidence that long-chain omega-3 fatty acids acylated in cell membranes of cardiomyocytes are the main contributors to myocardial protection on ischemic insult.⁵ The omega-3 status in red blood cell membranes is a well-validated surrogate marker for cardiomyocyte membrane omega-3 content.⁶ Several prospective studies in Western populations reported significant decreased incident CHD, in particular sudden cardiac death, with

Received on: October 25, 2018; final version accepted on: January 20, 2019.

From the Department of Biochemistry and Biotechnology, Human Nutrition Unit, IISPV, Hospital Universitari Sant Joan de Reus, Rovira i Virgili University, Reus, Spain (C.P., S.G., J.M., J.S.-S., M.B.); CIBER de Fisiopatología de la Obesidad y la Nutrición (CIBEROBN), Instituto de Salud Carlos III, Madrid, Spain (C.P., A.S.-V., S.G., J.M., R.E., M.F., C.R., D.C., E.R., J.T., J.L., L.S.-M., O.C., E.M.A., J.S.-S., M.B.); Lipid Clinic, Department of Endocrinology and Nutrition, Institut d'Investigacions Biomèdiques August Pi Sunyer (IDIBAPS), Hospital Clinic (A.S.-V., E.R.) and Department of Internal Medicine, Hospital Clínic, IDIBAPS August Pi i Sunyer Biomedical Research Institute (R.E.), University of Barcelona, Spain; Cardiovascular Risk and Nutrition (Regicor Study Group), Hospital del Mar Research Institute (IMIM), Barcelona, Spain (M.F., O.C.); Department of Preventive Medicine and Public Health, University of Navarra, Pamplona, Spain (C.R., S.C.); IdiSNA, Navarra Institute for Health Research, Pamplona, Spain (C.R., S.C.); Department of Preventive Medicine, University of Valencia, Spain (D.C., E.M.A.); Department of Neurology, University Hospital Araba, Vitoria, Spain (J.T.); Department of Family Medicine, Unit Research, Distrito Sanitario Atención Primaria Sevilla, Spain (J.L.); and Department of Clinical Sciences, Research Institute of Biomedical and Health Sciences, University of Las Palmas de Gran Canaria, Spain (L.S.-M.).

The online-only Data Supplement is available with this article at <https://www.ahajournals.org/doi/suppl/10.1161/ATVBAHA.118.312073>.

Correspondence to Mònica Bulló, BSc, PhD, Human Nutrition Unit, Faculty of Medicine and Health Sciences, Universitat Rovira i Virgili, St/Sant Llorenç 21, 43201 Reus, Spain, email monica.bullo@urv.cat; or Jordi Salas-Salvadó, MD, PhD, Human Nutrition Unit, Faculty of Medicine and Health Sciences, Universitat Rovira i Virgili, St/Sant Llorenç 21, 43201 Reus, Spain, email jordi.salas@urv.cat

© 2019 American Heart Association, Inc.

Arterioscler Thromb Vasc Biol is available at <https://www.ahajournals.org/journal/atvb>

DOI: 10.1161/ATVBAHA.118.312073

Nonstandard Abbreviations and Acronyms

CHD	coronary heart disease
CVD	cardiovascular disease
MUFA	monounsaturated fatty acid
OR	odds ratio
PUFA	polyunsaturated fatty acid
SFA	saturated fatty acid
T2D	type 2 diabetes mellitus
VLCSFAs	very long chain saturated fatty acids

increasing omega-3 status in red blood cells, which ultimately fostered its potential as a CHD risk marker.⁷ Recent research on CHD and fatty acids expanded beyond omega-3 fatty acids, including fatty acids with absent or marginal endogenous synthesis, in particular essential fatty acids and trans fatty acids, as recently reviewed.⁸ In addition, 2 recent large epidemiological studies reported that subjects with high proportions of very long chain saturated fatty acid (VLCSFAs; C20:0, C22:0, and C24:0) in red blood cell membranes had lower risk of sudden cardiac arrest⁹ and tended to have lower risk of CHD.¹⁰

However, data on the fatty acid composition of blood cell membrane in relation to CHD incidence are mostly from US cohorts. Taking this into account, the present case-control study, nested in the framework of the PREDIMED trial (Prevención con Dieta Mediterránea), aimed to test for associations between baseline and 1-year changes in the levels of fatty acids of blood cell membranes with incident CHD in a Mediterranean population at high cardiovascular risk.

Materials and Methods

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Study Design and Participants

The present study used a paired-matched case-control design nested within the PREDIMED trial (ISRCTN35739639), a multicenter, single-blinded, controlled trial, conducted in Spanish primary healthcare centers. The PREDIMED trials design has been described in detail elsewhere.^{11,12} This was conducted in 7447 participants at high risk of cardiovascular disease (CVD). Eligible participants were community-dwelling men (55–80 years), and women (60–80 years), who fulfilled at least 1 out of 2 criteria: (1) type 2 diabetes mellitus (T2D) or (2) 3 or more acute coronary syndrome risk factors: current smoking; hypertension (blood pressure >140/90 mm Hg or treatment with anti-hypertensive drugs); LDL (low-density lipoprotein) cholesterol >160 mg/dL (or treatment with hypolipidemic drugs); HDL (high-density lipoprotein) <50 mg/dL (women) and <40 mg/dL (men); body mass index ≥ 25 kg/m²; or a family history of premature CHD. Exclusion criteria included: history of CVD, any severe chronic illness, or low predicted likelihood of changing dietary habits according to the stages of change model, among others. Participants were randomly allocated to a Mediterranean diet supplemented with Extra virgin olive oil; a Mediterranean diet supplemented with mixed nuts, or a control diet consisting of advice to reduce fat intake. The Institutional Review Boards of the recruitment centers approved the study protocol, and participants provided written informed consent.

For the present study, we selected CHD incident cases defined by clinical symptoms, electrocardiographic findings, and measurements of biochemical markers.^{13,14} We used 4 sources of information to identify end points: repeated contacts with participants, family physicians, yearly review of medical records, and consultation of the

National Death Index. All medical records related to end points were examined by the End Point Adjudication Committee, whose members were blind to intervention allocation. Only end points that were confirmed by the Adjudication Committee and that occurred between October 1, 2003, and December 1, 2010, were considered.¹¹ We identified a total of 280 incident CHD cases but only 136 cases had available blood cell samples at baseline. Of 136 cases, 27 were stable angina, 65 were unstable angina, and 44 were myocardial infarction. Two controls were selected for each case whereas cases and controls were matched on age, sex, body mass index, intervention group, and time of permanence in the study to the time event. Of those, 211 participants (70 cases and 141 controls) had available samples after 1 year of follow-up and were included in the 1-year change analyses.

Fatty Acid Composition in Blood Cell Membranes

Overnight fasting period (>10 hours) blood samples were obtained by venipuncture and were stored at -80°C until fatty acids analysis. The blood fatty acid profile was determined by gas chromatography as described elsewhere.¹⁵ In brief, cells contained in a 100 μL aliquot of ethylenediaminetetraacetic acid-collected blood were hemolysed and spun. The pellet was dissolved in 1 mL BF_3 methanol solution and heated to hydrolyze and methylate glycerophospholipid fatty acids. The fatty acid methyl esters were isolated by adding n-hexane and were separated by gas chromatography using an Agilent HP 7890 Gas Chromatograph equipped with a 30 $\text{m} \times 0.25$ $\mu\text{m} \times 0.25$ mm SupraWAX-280 capillary column (Teknokroma, Barcelona, Spain), an autosampler, and a flame ionization detector. The amount of each fatty acid was expressed as a percentage of the total identified fatty acids in the sample. As recently stated by Stark et al,¹⁶ expressing fatty acid data in percentage are common because it makes it easier to compare the complex interactions between fatty acids. Total saturated fatty acid (SFA) was the sum of the percentage of C14:0, C16:0, C18:0, C20:0, C22:0, and C24:0. Total monounsaturated fatty acid (MUFA) was the sum of C16:1n7cis, C16:1n7trans, C18:1n9cis, C18:1n9trans, C20:1n9, and C24:1n9. Total n-6 polyunsaturated fatty acid (PUFA) was the sum of C18:2n6, C20:2n6, C20:3n6, C20:4n6, C22:4n6, and C22:5n6. The omega-3 index was calculated as the sum of the percentages of C20:5n3 and C22:6n3. VLCSFAs were the sum of C20:0, C22:0, and C24:0. The method used to quantify the fatty acids has been cross-validated against the method used in the original definition of the omega-3 index, proposed by Harris and Von Schacky.¹⁵

Assessment of Other Variables

At baseline, a 47-item questionnaire about lifestyle variables, smoking status, medical history, and medication use was administered. Physical activity was assessed using a validated Spanish version of the Minnesota Leisure-Time Physical Activity Questionnaire.¹⁷ Waist circumference was measured midway between the lowest rib and the iliac crest using an anthropometric tape. Participants' triacylglycerol, total cholesterol, and HDL-C were measured using fasting plasma at baseline. LDL-C levels were calculated by Friedwald formula whenever triacylglycerols were inferior to 300 mg/d. Serum inflammatory markers including IFN (interferon)- γ and ILs (interleukins) IL-1b, IL-6, IL-8, IL-10 were determined using a MILLIPLEX MAP Plex Kit (Merck Millipore, Billerica, MA). T2D was considered to be present at baseline by clinical diagnosis or use of antidiabetic medication.

Statistical Analysis

Baseline characteristics of cases and controls were described as means and SD (normally distributed continuous variables) or median and interquartile range (not normally distributed continuous variables) and percentages or numbers for categorical variables. The amount of each fatty acid is expressed as a percentage of the total identified fatty acids in the sample. A correlation (Spearman) matrix of the fatty acids under study at baseline was visualized through a heat map (R statistical package version 3.1.1; R Development Core Team, 2011; <http://cran.r-project.org>). The levels of fatty acids and total SFA, VLCSFAs, total MUFA, total n-6 PUFA, LCn-3PUFA,

and Omega-3 index were scaled to multiples of 1 SD. To estimate the association between fatty acids and incident CHD, we used conditional logistic regression model (conditional on the matching). An unadjusted-model and a model adjusted for T2D (yes/no) and smoking (never, current, and former) were fitted. We also examined the associations of 1-year changes in fatty acids with CHD incidence. With respect to fatty acids, we first calculated the difference between 1-year and baseline value and then scaled these differences to multiples of 1 SD. Two-sided *P* values were reported according to an α level=0.0017 ($\alpha=0.05$ with Bonferroni correction for 28 independent tests [including 22 individual fatty acids and total SFA, VLCSFAs, total MUFA, total n-6 PUFA, LCn-3PUFA, and Omega-3 index]). Statistical analyses were performed using Stata 14.1 (Stata Corp).

Results

Baseline characteristics of CHD cases and controls are shown in Table 1. At baseline, participants with incident CHD had a significantly higher T2D prevalence and were more likely to be current smokers compared with controls. Comparisons of fatty acids between cases and controls are displayed in Table I in the [online-only Data Supplement](#). Levels of VLCSFAs, especially C22:0 and C24:0 as well as C22:4n6, C22:5n6, and C24:1n9 were significantly lower in cases than in controls whereas levels of C18:1n9cis and C20:1n9 were higher in cases. Spearman correlation coefficients between baseline and 1-year measures of fatty acids are shown in Table II in the [online-only Data Supplement](#). Figure I in the [online-only Data Supplement](#) depicts the correlation matrix of the fatty acids under study at baseline. C22:0 was highly correlated with and C24:0 (Spearman's rank correlation coefficient [*r_s*]=0.87), while C20:1n9 was negatively correlated with these fatty acids (*r_s*=−0.31 and *r_s*=−0.47, respectively). Spearman rank correlations between C22:0, C24:0, VLCSFAs, C20:1n9, and certain food groups are presented in Table III in the [online-only Data Supplement](#). C22:0, C24:0, and VLCSFAs were negatively correlated with nuts consumption, whereas C20:1n9 was positively correlated with fish and olive oil consumption and negatively with dairy products.

Baseline and 1-Year Changes in Fatty Acids and CHD

Table 2 displays results for the analysis of baseline fatty acids with 136 incident CHD. After adjusting for potential confounders and multiple testing, 1 SD increase in C20:1n9 was associated with increased risk of CHD (odds ratio [OR], 1.58 [95% CI, 1.25–2.00, *P*<0.001]). We also found that 1 SD increase in VLCSFAs, C22:0, and C24:0 was significantly associated with decreased risk of CHD with ORs 0.44 (95% CI, 0.28–0.69), 0.41 (95% CI, 0.25–0.65), and 0.45 (95% CI 0.29, 0.70), respectively. Significant associations were also observed between several other fatty acids including C16:1n7trans, C18:1n9cis, C22:5n6, C24:1n9, VLCSFAs, and total MUFA but accounting for multiple comparisons, none of these associations remained statistically significant. No significant associations between 1-year changes in fatty acid levels with CHD incidence were found (Table 3).

In cross-sectional analyses adjusted for age (years), smoking (never, current, or former), body mass index (kg/m²), alcohol intake, leisure-time physical activity (metabolic equivalent tasks in minutes per day), and T2D, C22:0 was inversely correlated with IL-10 with Spearman partial correlation coefficients

Table 1. Comparison of Coronary Heart Disease Risk Factors Between Case and Control Subjects at Baseline*

	Cases	Controls	<i>P</i> Value
n	136	272	
Age, y	67.8±6.4	67.4±6.2	0.593
Sex (women), %	39.7	41.2	0.776
BMI, kg/m ²	29.5±3.2	29.5±3.3	0.908
WC, cm	100.9±8.9	101.0±8.6	0.938
Physical activity, METs/d	209.0 (88.2±362.4)	223.0 (88.0±422.5)	0.330
Intervention group, %			
MedDiet + EVOO	31.6	28.7	0.818
MedDiet + nuts	34.5	36.7	
Control group	33.8	34.5	
Type 2 diabetes mellitus, %	63.2	48.9	0.006
Hypertension, %	82.3	82.7	0.926
Dyslipidemia, %	63.9	71.7	0.112
Smoking, %			
Never	44.1	53.7	0.010
Former	32.3	34.2	
Current	23.5	12.1	
Total cholesterol, mg/dL	207.6±35.5	209.2±41.4	0.710
HDL cholesterol, mg/dL	48.5±8.9	50.9±14.8	0.093
LDL cholesterol, mg/dL	133.5±37.8	132.6±36.8	0.899
Triacylglycerol, mg/dL	155.7±76.3	154.4±111.0	0.940
IFN- γ	15.8 (10.3±23.0)	15.8 (10.0±23.1)	0.701
IL-1b	2.0 (1.1±2.9)	1.9 (1.3±2.7)	0.971
IL-6	2.9 (2.2±4.3)	3.2 (1.9±4.3)	0.915
IL-8	9.5 (7.1±13.9)	9.0 (6.8±13.8)	0.226
IL-10	21.6 (10.1±31.1)	19.6 (10.1±32.7)	0.940

Values are means±SDs unless otherwise indicated. The χ^2 test was used for comparison of categorical variables and Student *t* test or Mann-Whitney *U* test was used for comparison of continuous variables.

BMI indicates body mass index; EVOO, extra-virgin olive oil; HDL, high-density lipoprotein; IFN- γ , interferon- γ ; IL, interleukin; LDL, low-density lipoprotein; MedDiet, Mediterranean diet; MET, metabolic equivalent; and WC, waist circumference.

*Case and control subjects were matched on age, sex, body mass index, and intervention group and time of permanence in the study to the time event.

(*r_s*) of −0.16. A similar trend was observed between C24:0 and IL-10 but did not reach the significant level (*r_s*=−0.14). No significant correlations were observed between these 2 VLCSFAs and other inflammatory markers (IFN- γ , IL-1b, IL-6, and IL-8). The C20:1n9 fatty acid was not significantly correlated with any of the aforementioned inflammatory markers. Notably, C22:0 was also inversely correlated with 1-year changes in levels of IL-10 (*r_s*=−0.16, *P*=0.030) after further adjustment for baseline IL-10. Further cross-sectional analyses adjusted for age (years), sex (men or women), body mass index (kg/m²), smoking (never, current, or former), leisure-time physical activity (metabolic equivalent tasks in minutes per day), dyslipidemia

Table 2. ORs and 95% CIs for Incident Coronary Heart Disease Associated With 1 SD Increment in Baseline Fatty Acids in Blood Cell Membranes in the PREDIMED Trial, 2003–2010

Fatty Acid	Model 1	Model 2	P Value Model 1	P Value Model 2
	OR (95% CI)	OR (95% CI)		
C14:0	0.73 (0.54–0.98)	0.74 (0.55–0.98)	0.040	0.036
C16:0	0.99 (0.79–1.23)	0.93 (0.74–1.18)	0.559	0.935
C16:1n7cis	1.12 (0.91–1.38)	1.11 (0.89–1.37)	0.274	0.356
C16:1n7trans	1.16 (0.95–1.42)	1.15 (0.93–1.42)	0.129	0.190
C18:0	0.99 (0.80–1.24)	0.97 (0.77–1.23)	0.821	0.988
C18:1n9cis	1.41 (1.12–1.77)	1.36 (1.08–1.72)	0.003	0.010
C18:1n9trans	1.21 (0.96–1.51)	1.16 (0.91–1.48)	0.103	0.214
C18:2n6	0.99 (0.80–1.24)	1.08 (0.86–1.35)	0.983	0.496
C18:3n3	1.16 (0.94–1.43)	1.16 (0.94–1.43)	0.278	0.165
C20:0	1.01 (0.82–1.25)	1.01 (0.81–1.25)	0.907	0.928
C20:1n9	1.64 (1.31–2.07)	1.58 (1.25–2.00)	<0.001*	<0.001*
C20:2n6	1.05 (0.86–1.29)	1.04 (0.85–1.29)	0.599	0.660
C20:3n6	1.14 (0.91–1.42)	1.22 (0.97–1.55)	0.241	0.088
C20:4n6	0.93 (0.75–1.15)	0.94 (0.75–1.17)	0.503	0.574
C20:5n3	1.18 (0.96–1.45)	1.20 (0.97–1.48)	0.121	0.096
C22:0	0.40 (0.26–0.63)	0.44 (0.28–0.69)	<0.001*	<0.001*
C22:4n6	0.72 (0.57–0.91)	0.73 (0.58–0.93)	0.006	0.011
C22:5n6	0.63 (0.49–0.82)	0.66 (0.51–0.86)	0.001	0.002
C22:5n3	0.90 (0.73–1.12)	0.92 (0.73–1.15)	0.349	0.454
C22:6n3	1.01 (0.81–1.24)	1.05 (0.84–1.32)	0.942	0.642
C24:0	0.38 (0.24–0.60)	0.41 (0.25–0.65)	<0.001*	<0.001*
C24:1n9	0.62 (0.48–0.81)	0.66 (0.50–0.86)	0.001	0.003
Total SFA	0.92 (0.73–1.15)	0.87 (0.68–1.11)	0.475	0.268
VLCSFAs	0.42 (0.27–0.65)	0.45 (0.29–0.70)	<0.001*	<0.001*
Total MUFA	1.35 (1.08–1.69)	1.31 (1.04–1.65)	0.007	0.020
Total n-6 PUFA	0.90 (0.72–1.13)	0.94 (0.74–1.20)	0.379	0.650
LCn-3PUFA	1.01 (0.82–1.25)	1.05 (0.84–1.32)	0.915	0.657
Omega-3 index	1.04 (0.84–1.29)	1.09 (0.87–1.36)	0.700	0.452

Conditional logistic regression analysis was used. Model 1 is unadjusted. Model 2 adjusted for smoking and diabetes mellitus status that were significantly different between cases (n=136) and controls (n=272). MUFA indicates monounsaturated fatty acid; OR, odds ratio; PREDIMED, Prevención con Dieta Mediterránea; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; and VLC, very long chain.

*Remained significant after Bonferroni correction for multiple comparisons.

(yes or no), and hypertension (yes or no) revealed significant inverse associations of VLCSFAs and C24:0 with T2D (OR, 0.43 [95% CI, 0.23–0.82]) and (OR, 0.35 [95% CI, 0.17–0.70]), respectively. A similar trend was observed for C22:0 (OR, 0.62 [95% CI, 0.36–1.06]).

Discussion

In this case-control study within the PREDIMED trial, we aimed to identify fatty acids in blood cells potentially related to CHD in 408 older adults at high CVD risk. We observed that baseline levels of VLCSFAs and especially C22:0, C24:0 showed significant inverse associations with CHD incidence,

independently of smoking and T2D. No significant associations were observed for 1-year changes.

Identifying new biomarkers aiding to reclassify the risk of CHD in populations with a high burden of cardiovascular risk factors is an emerging field in public health. In this regard, our findings reinforce the suitability of blood cell membrane VLCSFAs, in particular C22:0 and C24:0, for this purpose. In addition, our findings suggest that not all SFAs are equal in relation to health and disease, hence contributing to questioning the long-standing dogma of the adverse effects of all types of SFA, regardless of their origin or chemical characteristics.¹⁸

Table 3. ORs and 95% CIs for Incident Coronary Heart Disease Associated With 1 SD Increment in 1-Year Changes in Fatty Acids in Blood Cell Membranes in the PREDIMED Trial, 2003–2010

Fatty Acid	Model 1	Model 2	P Value Model 1	P Value Model 2
	OR (95% CI)	OR (95% CI)		
C14:0	1.24 (0.93–1.65)	1.29 (0.93–1.79)	0.148	0.123
C16:0	1.06 (0.78–1.44)	1.04 (0.75–1.43)	0.682	0.820
C16:1n7cis	0.86 (0.65–1.14)	0.81 (0.60–1.10)	0.312	0.186
C16:1n7trans	0.97 (0.73–1.31)	0.91 (0.66–1.25)	0.871	0.562
C18:0	1.08 (0.79–1.48)	1.11 (0.79–1.54)	0.623	0.546
C18:1n9cis	1.04 (0.78–1.40)	1.02 (0.74–1.38)	0.767	0.914
C18:1n9trans	0.77 (0.56–1.06)	0.79 (0.56–1.12)	0.110	0.192
C18:2n6	0.87 (0.63–1.18)	0.88 (0.63–1.22)	0.365	0.439
C18:3n3	1.00 (0.75–1.33)	1.02 (0.75–1.39)	0.990	0.869
C20:0	1.00 (0.75–1.35)	1.04 (0.76–1.42)	0.973	0.798
C20:1n9	0.91 (0.68–1.22)	0.96 (0.71–1.30)	0.529	0.811
C20:2n6	0.87 (0.65–1.17)	0.94 (0.69–1.28)	0.356	0.695
C20:3n6	0.94 (0.70–1.25)	0.96 (0.71–1.31)	0.673	0.805
C20:4n6	0.93 (0.68–1.26)	0.95 (0.69–1.32)	0.640	0.782
C20:5n3	1.19 (0.88–1.61)	1.18 (0.86–1.60)	0.254	0.300
C22:0	1.45 (0.67–3.11)	1.32 (0.64–2.73)	0.342	0.444
C22:4n6	0.97 (0.71–0.32)	1.00 (0.72–1.39)	0.868	0.987
C22:5n6	1.07 (0.82–1.41)	1.06 (0.79–1.43)	0.595	0.674
C22:5n3	0.93 (0.68–1.26)	0.93 (0.67–1.29)	0.634	0.683
C22:6n3	0.89 (0.65–1.22)	0.88 (0.63–1.23)	0.473	0.459
C24:0	1.37 (0.77–2.43)	1.27 (0.72–2.25)	0.279	0.411
C24:1n9	1.12 (0.79–1.60)	1.12 (0.75–1.65)	0.524	0.575
Total SFA	1.13 (0.82–1.55)	1.11 (0.79–1.55)	0.451	0.530
VLCSFAs	1.37 (0.76–2.45)	1.29 (0.70–2.37)	0.291	0.418
Total MUFA	1.03 (0.77–1.38)	1.00 (0.74–1.37)	0.828	0.978
Total n-6 PUFA	0.91 (0.66–1.24)	0.93 (0.67–1.30)	0.546	0.687
LCn-3PUFA	0.93 (0.68–1.27)	0.92 (0.66–1.28)	0.646	0.638
Omega-3 index	0.93 (0.68–1.27)	0.92 (0.66–1.28)	0.656	0.632

Conditional logistic regression analysis was used. Model 1 is unadjusted. Model 2 adjusted for smoking and diabetes mellitus status that were significantly different between cases (n=70) and controls (n=141). MUFA indicates monounsaturated fatty acid; OR, odds ratio; PREDIMED, Prevención con Dieta Mediterránea; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; and VLC, very long chain.

Our results are in agreement with a recent report from the Nurses' Health Study and the Health Professionals Follow-up Study, in which higher levels of VLCSFAs in plasma and red blood cells were associated with lower risk of CHD.¹⁰ Inverse associations between VLCSFAs in plasma phospholipids and incident atrial fibrillation,¹⁹ and between VLCSFAs in red blood cells and sudden cardiac arrest⁹ have also been reported. Our study broadens these studies by extending the findings to an older population at high cardiovascular risk while reproducing them in a much different geographic setting.

The biological mechanisms underlying these observations remain elusive. Most data on circulating fatty acids and CVD relate to fatty acids with absent or marginal endogenous

synthesis, namely essential fatty acids, trans fatty acids, and long-chain omega-3 fatty acids,⁸ for which membrane status is considered an optimal surrogate marker of their dietary intake.²⁰

Circulating VLCSFAs may have both dietary and metabolic origin. The former is marginal, being the sources mostly macadamia nuts and peanuts.²¹ In our study, VLCSFAs were negatively correlated with nuts consumption and, therefore, circulating VLCSFAs could not be considered as markers of nuts consumption. In contrast, VLCSFAs are easily obtained from the elongation of stearic acid by the action of the elongase *elov11*, an enzyme closely linked to the metabolism of sphingomyelins and ceramides.²² These lipids are found in high concentrations within cell membranes and play an

important role in membrane function and cellular signaling.²³ Specifically, the VLCSCFA content in sphingomyelin may influence the structure/function of lipid rafts, sphingomyelin-rich membrane domains where ion channels and signaling molecules are located. In addition, experimental evidence suggests that the VLCSCFA content in ceramides has a profound effect on ceramide-induced apoptosis.²⁴ According to previous animal and cell culture studies, the balance between C16:0 and VLCSCFA containing ceramides seems to be the key factor for the induction of apoptosis.²⁵ Whether C22:0 and C24:0 may lower the risk of metabolic dysfunction and CHD by lowering endogenous levels of ceramides containing shorter SFA²⁵ is unknown. Interestingly, we found a favorable association between VLCSFAs, C24:0, and T2D which is in line with previous studies^{26,27} and we could speculate that VLCSFAs and especially C24:0 may lower endogenous levels of ceramides containing shorter SFA.^{28,29} However, C22:0 was favorably correlated with a marker of inflammation, IL-10, which has been associated with an increased risk for future cardiovascular events.^{30–32} Further studies are needed to examine this hypothesis, in particular focusing on whether circulating VLCSCFA can be modulated through diet.³³

Another member of long-chain fatty acids, C20:1n9 was found to be associated with higher CHD incidence. However, in a previous study, the consumption of macadamia nuts raised plasma concentrations of C20:1n9 and favorably influenced oxidative stress, thrombosis, and inflammation.³⁴ This long chain monounsaturated fatty acid (LCMUFA) can be derived from the diet³⁵ and in the present study was directly correlated with both consumption of fish and total olive oil. After adjusting the analyses for fish and total olive oil, our results changed slightly (data not shown) suggesting that the association between this type of LCMUFA and CHD may not be mediated by these foods. Because this LCMUFA can also be formed by de novo synthesis, by the action of fatty acid elongases on oleic acid (C18:1n9),³⁶ whether higher levels of C20:1n9 in those who developed CHD as compared to the controls indicates increased elongase activity is unknown and requires further work hypotheses and research to be determined.

Contrary to previous studies,^{37–39} we did not find any significant association between omega-3 fatty acids and CHD. Recent studies highlight that there is moderate strength of evidence that marine oil supplementation lowers risk of major adverse cardiovascular events and low strength of evidence that higher marine oil intake is associated with lower risk of CHD and congestive heart failure.⁴⁰ Furthermore, moderate- and high-quality evidence suggests that increasing eicosapentaenoic acid and docosahexaenoic acid has little or no effect on cardiovascular health (evidence mainly from supplement trials).⁴¹

The results of the present study should be interpreted in the context of its limitations and strengths. First, this is an observational study and causality cannot be established. Second, although we adjusted for T2D and smoking, we cannot exclude the role of residual confounding by unknown factors which weakens our ability to draw causal conclusions. Third, participants were elderly Mediterranean individuals at high cardiovascular risk, and this may limit the generalizability of the findings to other age groups or populations. Strengths

include the prospective design and objective measurement of twenty-two individual fatty acids in blood cell membranes.

In conclusion, the results of this study suggest that among older Mediterranean adults at high CVD risk, higher levels of C22:0 and C24:0 are associated with a lower CHD incidence, whereas higher levels of C20:1n9 are associated with higher risk. This study adds to the growing body of evidence, suggesting potential differences in the CVD effects of different types of circulating SFAs. The potential mechanisms linking the aforementioned fatty acids and incident CHD must also be further investigated.

Sources of Funding

This study was funded by Fundació La Marató de TV3 (201512.30.31.32). C. Papandreou is supported by a postdoctoral fellowship granted by the Autonomous Government of Catalonia (PERIS 2016–2020 Incorporació de Científics i Tecnòlegs, SLT002/0016/00428). A. Sala-Vila holds a Miguel Servet contract (CP12/03299, Instituto de Salud Carlos III, Spain). J. Muralidharan has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No. 713679 and from the Universitat Rovira i Virgili (URV).

Disclosures

None.

References

1. GBD 2016 Causes of Death Collaborators. Global, regional, and national age-sex specific mortality for 264 causes of death, 1980–2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet*. 2017;390:1151–1210. doi: 10.1016/S0140-6736(17)32152-9
2. Piepoli MF, Hoes AW, Agewall S, et al; ESC Scientific Document Group. 2016 European Guidelines on cardiovascular disease prevention in clinical practice: the Sixth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of 10 societies and by invited experts) Developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). *Eur Heart J*. 2016;37:2315–2381. doi: 10.1093/eurheartj/ehw106
3. Khot UN, Khot MB, Bajzer CT, Sapp SK, Ohman EM, Brener SJ, Ellis SG, Lincoff AM, Topol EJ. Prevalence of conventional risk factors in patients with coronary heart disease. *JAMA*. 2003;290:898–904. doi: 10.1001/jama.290.7.898
4. Maulucci G, Cohen O, Daniel B, Sansone A, Petropoulou PI, Filou S, Spyridonidis A, Pani G, De Spirito M, Chatgilliloglu C, Ferreri C, Kypreos KE, Sasson S. Fatty acid-related modulations of membrane fluidity in cells: detection and implications. *Free Radic Res*. 2016;50(sup1):S40–S50. doi: 10.1080/10715762.2016.1231403
5. McLennan PL. Cardiac physiology and clinical efficacy of dietary fish oil clarified through cellular mechanisms of omega-3 polyunsaturated fatty acids. *Eur J Appl Physiol*. 2014;114:1333–1356. doi: 10.1007/s00421-014-2876-z
6. Harris WS, Sands SA, Windsor SL, Ali HA, Stevens TL, Magalski A, Porter CB, Borkon AM. Omega-3 fatty acids in cardiac biopsies from heart transplantation patients: correlation with erythrocytes and response to supplementation. *Circulation*. 2004;110:1645–1649. doi: 10.1161/01.CIR.0000142292.10048.B2
7. Harris WS. The omega-3 index: from biomarker to risk marker to risk factor. *Curr Atheroscler Rep*. 2009;11:411–417.
8. Jackson KH, Harris WS. Blood fatty acid profiles: new biomarkers for cardiometabolic disease risk. *Curr Atheroscler Rep*. 2018;20:22. doi: 10.1007/s11883-018-0722-1
9. Lemaitre RN, King IB, Rice K, McKnight B, Sotoodehnia N, Rea TD, Johnson CO, Raghunathan TE, Cobb LA, Mozaffarian D, Siscovick DS. Erythrocyte very long-chain saturated fatty acids associated with lower risk of incident sudden cardiac arrest. *Prostaglandins Leukot Essent Fatty Acids*. 2014;91:149–153. doi: 10.1016/j.plefa.2014.07.010

10. Malik VS, Chiuve SE, Campos H, Rimm EB, Mozaffarian D, Hu FB, Sun Q. Circulating very-long-chain saturated fatty acids and incident coronary heart disease in US men and women. *Circulation*. 2015;132:260–268. doi: 10.1161/CIRCULATIONAHA.114.014911
11. Estruch R, Ros E, Salas-Salvadó J, et al; PREDIMED Study Investigators. Primary prevention of cardiovascular disease with a Mediterranean diet supplemented with extra-virgin olive oil or nuts. *N Engl J Med*. 2018;378:e34. doi: 10.1056/NEJMoa1800389
12. Martínez-González MÁ, Corella D, Salas-Salvadó J, et al; PREDIMED Study Investigators. Cohort profile: design and methods of the PREDIMED study. *Int J Epidemiol*. 2012;41:377–385. doi: 10.1093/ije/dyq250
13. Alpert JS, Thygesen K, Antman E, Bassand JP. Myocardial infarction redefined—a consensus document of The Joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infarction. *J Am Coll Cardiol*. 2000;36:959–969.
14. Cannon CP, Braunwald E. Unstable angina and non-ST elevation myocardial infarction. In: *Braunwald's Heart Disease. A Textbook of Cardiovascular Medicine*. Philadelphia, PA: Elsevier Saunders; 2005:1243–1280.
15. Sala-Vila A, Harris WS, Cofán M, Pérez-Heras AM, Pintó X, Lamuela-Raventós RM, Covas MI, Estruch R, Ros E. Determinants of the omega-3 index in a Mediterranean population at increased risk for CHD. *Br J Nutr*. 2011;106:425–431. doi: 10.1017/S0007114511000171
16. Stark KD, Van Elswyk ME, Higgins MR, Weatherford CA, Salem N Jr. Global survey of the omega-3 fatty acids, docosahexaenoic acid and eicosapentaenoic acid in the blood stream of healthy adults. *Prog Lipid Res*. 2016;63:123–152. doi: 10.1016/j.plipres.2016.05.001
17. Elosua R, Marrugat J, Molina L, Pons S, Pujol E. Validation of the Minnesota leisure time physical activity questionnaire in Spanish men. The MARATHOM Investigators. *Am J Epidemiol*. 1994;139:1197–1209.
18. Dawczynski C, Kleber ME, März W, Jahreis G, Lorkowski S. Saturated fatty acids are not off the hook. *Nutr Metab Cardiovasc Dis*. 2015;25:1071–1078. doi: 10.1016/j.numecd.2015.09.010
19. Fretts AM, Mozaffarian D, Siscovick DS, Djousse L, Heckbert SR, King IB, McKnight B, Sitlani C, Sacks FM, Song X, Sotoodehnia N, Spiegelman D, Wallace ER, Lemaitre RN. Plasma phospholipid saturated fatty acids and incident atrial fibrillation: the Cardiovascular Health Study. *J Am Heart Assoc*. 2014;3:e000889. doi: 10.1161/JAHA.114.000889
20. Hodson L, Skeaff CM, Fielding BA. Fatty acid composition of adipose tissue and blood in humans and its use as a biomarker of dietary intake. *Prog Lipid Res*. 2008;47:348–380. doi: 10.1016/j.plipres.2008.03.003
21. United States Department of Agriculture, Agricultural Research Service. USDA National Nutrient Database for Standard Reference: Release 27 [Internet] 2015. <https://www.ars.usda.gov/northeast-area/beltsville-md-bhnrc/beltsville-human-nutrition-research-center/nutrient-data-laboratory/docs/usda-national-nutrient-database-for-standard-reference/>.
22. Ohno Y, Suto S, Yamanaka M, Mizutani Y, Mitsutake S, Igarashi Y, Sassa T, Kihara A. ELOVL1 production of C24 acyl-CoAs is linked to C24 sphingolipid synthesis. *Proc Natl Acad Sci USA*. 2010;107:18439–18444. doi: 10.1073/pnas.1005572107
23. Milhas D, Clarke CJ, Hannun YA. Sphingomyelin metabolism at the plasma membrane: implications for bioactive sphingolipids. *FEBS Lett*. 2010;584:1887–1894. doi: 10.1016/j.febslet.2009.10.058
24. Pewzner-Jung Y, Ben-Dor S, Futerman AH. When do Lasses (longevity assurance genes) become CerS (ceramide synthases)? insights into the regulation of ceramide synthesis. *J Biol Chem*. 2006;281:25001–25005. doi: 10.1074/jbc.R600010200
25. Grösch S, Schiffmann S, Geisslinger G. Chain length-specific properties of ceramides. *Prog Lipid Res*. 2012;51:50–62. doi: 10.1016/j.plipres.2011.11.001
26. Forouhi NG, Koulman A, Sharp SJ, et al. Differences in the prospective association between individual plasma phospholipid saturated fatty acids and incident type 2 diabetes: the EPIC-InterAct case-cohort study. *Lancet Diabetes Endocrinol*. 2014;2:810–818. doi: 10.1016/S2213-8587(14)70146-9
27. Lemaitre RN, Fretts AM, Sitlani CM, Biggs ML, Mukamal K, King IB, Song X, Djousse L, Siscovick DS, McKnight B, Sotoodehnia N, Kizer JR, Mozaffarian D. Plasma phospholipid very-long-chain saturated fatty acids and incident diabetes in older adults: the Cardiovascular Health Study. *Am J Clin Nutr*. 2015;101:1047–1054. doi: 10.3945/ajcn.114.101857
28. Chavez JA, Summers SA. A ceramide-centric view of insulin resistance. *Cell Metab*. 2012;15:585–594. doi: 10.1016/j.cmet.2012.04.002
29. Kusminski CM, Shetty S, Orzi L, Unger RH, Scherer PE. Diabetes and apoptosis: lipotoxicity. *Apoptosis*. 2009;14:1484–1495. doi: 10.1007/s10495-009-0352-8
30. Lakoski SG, Liu Y, Brosnihan KB, Herrington DM. Interleukin-10 concentration and coronary heart disease (CHD) event risk in the estrogen replacement and atherosclerosis (ERA) study. *Atherosclerosis*. 2008;197:443–447. doi: 10.1016/j.atherosclerosis.2007.06.033
31. Welsh P, Murray HM, Ford I, Trompet S, de Craen AJ, Jukema JW, Stott DJ, McInnes IB, Packard CJ, Westendorp RG, Sattar N; PROSPER Study Group. Circulating interleukin-10 and risk of cardiovascular events: a prospective study in the elderly at risk. *Arterioscler Thromb Vasc Biol*. 2011;31:2338–2344. doi: 10.1161/ATVBAHA.111.231795
32. Yilmaz MI, Solak Y, Saglam M, Cayci T, Acikel C, Unal HU, Eyiletlen T, Oguz Y, Sari S, Carrero JJ, Stenvinkel P, Covic A, Kanbay M. The relationship between IL-10 levels and cardiovascular events in patients with CKD. *Clin J Am Soc Nephrol*. 2014;9:1207–1216. doi: 10.2215/CJN.08660813
33. Lauritzen L, Hellgren LI. Plasma phospholipid very-long-chain saturated fatty acids: a sensitive marker of metabolic dysfunction or an indicator of specific healthy dietary components? *Am J Clin Nutr*. 2015;101:901–902. doi: 10.3945/ajcn.115.110569
34. Garg ML, Blake RJ, Wills RB, Clayton EH. Macadamia nut consumption modulates favourably risk factors for coronary artery disease in hypercholesterolemic subjects. *Lipids*. 2007;42:583–587. doi: 10.1007/s11745-007-3042-8
35. Chowdhury K, Banu LA, Khan S, Latif A. Studies on the fatty acid composition of edible oil. *Bangladesh J Sci Ind Res*. 2007;42:311–316.
36. Suburu J, Gu Z, Chen H, Chen W, Zhang H, Chen YQ. Fatty acid metabolism: implications for diet, genetic variation, and disease. *Food Biosci*. 2013;4:1–12. doi: 10.1016/j.fbio.2013.07.003
37. Siscovick DS, Raghunathan TE, King I, Weinmann S, Wicklund KG, Albright J, Bovbjerg V, Arbogast P, Smith H, Kushi LH. Dietary intake and cell membrane levels of long-chain n-3 polyunsaturated fatty acids and the risk of primary cardiac arrest. *JAMA*. 1995;274:1363–1367.
38. Block RC, Harris WS, Reid KJ, Sands SA, Spertus JA. EPA and DHA in blood cell membranes from acute coronary syndrome patients and controls. *Atherosclerosis*. 2008;197:821–828. doi: 10.1016/j.atherosclerosis.2007.07.042
39. Block RC, Harris WS, Reid KJ, Spertus JA. Omega-6 and trans fatty acids in blood cell membranes: a risk factor for acute coronary syndromes? *Am Heart J*. 2008;156:1117–1123. doi: 10.1016/j.ahj.2008.07.014
40. Balk EM, Lichtenstein AH. Omega-3 fatty acids and cardiovascular disease: summary of the 2016 agency of healthcare research and quality evidence review. *Nutrients*. 2017;9:E865.
41. Abdelhamid AS, Brown TJ, Brainard JS, Biswas P, Thorpe GC, Moore HJ, Deane KH, AlAbdulghafoor FK, Summerbell CD, Worthington HV, Song F, Hooper L. Omega-3 fatty acids for the primary and secondary prevention of cardiovascular disease. *Cochrane Database Syst Rev*. 2018;7:CD003177.

Highlights

- Coronary heart disease is the leading cause of death worldwide.
- This study used a paired-matched case-control design nested within the PREDIMED trial (Prevención con Dieta Mediterránea) to test for associations between baseline and 1-year changes in the levels of fatty acids of blood cell membranes with incident coronary heart disease.
- Higher levels of C22:0 and C24:0 were associated with a lower, whereas higher levels of C20:1n9 were associated with a higher coronary heart disease risk.
- This study adds to the growing body of evidence suggesting potential differences in the cardiovascular disease effects of different types of circulating saturated fatty acids.