ORIGINAL RESEARCH

Plasma Phospholipid Fatty Acid Biomarkers of Dietary Fat Quality and Endogenous Metabolism Predict Coronary Heart Disease Risk: A Nested Case-Control Study Within the Women's Health Initiative Observational Study

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Background—Although the relationship between dietary fat quality and coronary heart disease (CHD) risk has been evaluated, typically using diet questionnaires, results are inconsistent and data in postmenopausal women are limited. Plasma phospholipid fatty acid (PL-FA) profiles, reflecting dietary intake and endogenous FA metabolism, may better predict diet–CHD risk.

Methods and Results—Using a nested case-control design, we assessed the association between plasma PL-FA profiles and CHD risk in 2448 postmenopausal women (1224 cases with confirmed CHD and 1224 controls matched for age, enrollment date, race/ethnicity, and absence of CHD at baseline and after 4.5 years of follow-up) participating in the Women's Health Initiative observational study. PL-FA profile was measured using gas chromatography. Product/precursor ratios were used to estimate stearoyl-CoA-desaturase (16:1n-7/16:0, 18:1n-9/18:0), Δ6-desaturase (20:3n-6/18:2n-6), and Δ5-desaturase (20:4n-6/20:3n-6) activities, indicators of endogenous FA metabolism. Multivariate conditional logistic regression was used to obtain odds ratios (95% CIs) for CHD risk. While no associations were observed for the predominant PL fatty acid (16:0, 18:0, 18:1n-9, and 18:2n-6), plasma PL–saturated fatty acid (1.20 [1.08 to 1.32]) and endogenously synthesized PL ω6 fatty acids (20:3n-6; 3.22 [1.95 to 5.32]), 22:5n-6; 1.63 [1.20 to 2.23]) and Δ6-desaturase (1.25 [1.11 to 1.41]) were positively associated with CHD risk. PL-ω3 fatty acids (20:5n-3; 0.73 [0.58 to 0.93], 22:5n-3; 0.56 [0.33 to 0.94], 22:6n-3; 0.56 [0.39 to 0.80]), 18:1n-7 (0.54 [0.29 to 0.99]), and Δ5-desaturase (0.78 [0.70 to 0.88]) were inversely associated with CHD risk. Results support current guidelines regarding regular fish consumption. Additional findings include associations between endogenously synthesized fatty acids and CHD risk, which were partly explained by changes in Δ6-desaturase and Δ5-desaturase indexes, suggesting that in vivo metabolism may also play an important role in predicting CHD risk in this cohort of postmenopausal women.

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Cardiovascular disease (CVD) is the leading cause of morbidity and mortality in older women.[1] Dietary fat intake has long been implicated in the etiology of coronary heart disease (CHD).[2–4] However, there are limited and inconsistent data on the relationship between dietary fat quality and CHD risk, particularly for postmenopausal women.[5–7] Potential reasons for this discrepancy could be related to the subjective nature of the tools used to assess dietary intake and inherent limitations of the databases used to estimate fatty acid intakes.[8,9] For these reasons, fatty acids measured in various biological specimens are being increasingly used as objective indicators of dietary fat quality.[10–13] Of particular interest are the very long–chain ω3 fatty acids (eicosapentaenoic [20:5n-3], docosapentaenoic [22:5n-3], and docosahexaenoic [22:6n-3]), *trans*–fatty acids (18:1t and 18:2t), and odd-chain fatty acids (15:0 and 17:0) as biomarkers for fish,[14–16] partially-hydrogenated fat,[17,18] and dairy[19–22] intake, respectively. These fatty acids cannot be synthesized in vivo and thus provide the best estimates of intake.[13]

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However, in addition to dietary intake, circulating fatty acid concentrations reflect endogenous fatty acid metabolism: fatty acid synthesis (de novo lipogenesis [DNL]), and fatty acid desaturation/elongation.[23] Some evidence suggests that palmitic (16:0), stearic (18:0), palmitoleic (16:1n-7), hexadecanoic (16:1n-9), and *cis*-vaccenic (18:1n-7) fatty acids, which are partly synthesized endogenously by the DNL pathway from carbohydrates and proteins,[24] are associated with CHD risk, although results are mixed.[25–28] Likewise, limited evidence suggests that desaturase indexes, surrogate indicators of fatty acid desaturase enzyme activity, are associated with various metabolic disorders as well as CVD death.[29–31] Of note, the majority of studies to date examining the relationship between circulating fatty acid concentrations and CHD risk have focused on individual fatty acids.[14,32–35] Given that circulating fatty acid concentrations reflect in vivo fatty acid exposure, a consequence of bioavailability, postingestion metabolism by gut microbiota, enterohepatic circulation, nutrient interactions, tissue storage, turnover, metabolism, and excretion,[36] as well as the interdependency of individual fatty acids, measuring the fatty acid profile may be more biologically relevant and thus provide a more robust assessment of the relationship between dietary fat quality and CHD risk. A limited number of studies have investigated the association between CHD and the entire spectrum of fatty acids.[25,37–39] Among these studies, different blood lipid fractions (erythrocytes, adipose tissue, serum/plasma including cholesteryl ester [CE], triglyceride [TG], and phospholipid [PL]) fractions were used to measure the fatty acid profile. This precludes making direct comparisons among studies due to the selective accumulation of individual fatty acids in these fractions. While the fatty acid profile of adipose tissue TG is considered a good indicator of long-term dietary fat quality in weight-stable individuals due to its slow turnover time,[40–43] the invasive nature of sample collection precludes routine use. Analysis of the fatty acid profile of the PL or CE fraction is considered to be a better indicator of medium-term intake than the TG fraction, the latter reflecting shorter-term intake.[40,44] Studies that have assessed the PL fatty acid profile have primarily focused on men[25,37,38] or have reported combined results for men and women.[39,45] The present study used samples from postmenopausal women participating in the observational cohort of the Women's Health Initiative (WHI-OS) study to determine the association between plasma PL fatty acid profiles and CHD risk, with the intent to confirm and/or expand the database of knowledge for this high-risk and growing segment of the population.

Methods

The WHI-OS is a prospective cohort study designed to assess the impact of biological, lifestyle, biochemical, and genetic factors on cancer and other major health events, including CHD. The study enrolled 93 676 postmenopausal women between the ages of 50 and 79 years who were recruited to the WHI-OS at 40 clinical centers in the United States. A detailed description of the WHI-OS design has been published.[46,47]

Study Design and Population

A nested case-control study design was used. All locally confirmed cases of CHD (hospitalized myocardial infarction [MI], definite silent MI, and coronary death defined as death consistent with CHD as the underlying cause, based on review of medical records and death certificate) reported from the September 2005 database in the WHI-OS were selected for the original case sampling.[48] A total of 2468 cases were initially eligible. Potential cases were excluded based on the following hierarchical criteria: (1) lack of available baseline plasma sample (n=28), (2) lack of baseline food frequency questionnaire (n=126), and (3) CVD reported at baseline, where CVD is defined as MI, angina, coronary artery bypass graft surgery/percutaneous transluminal coronary angioplasty, carotid artery disease, congestive heart failure, stroke or peripheral vascular disease, or CHD death (n=765). Potential controls were excluded for all of these reasons as well as CVD occurring during follow-up (mean 4.5 years). Among the 1549 cases meeting the eligibility criteria, 1288 had a previously matched eligible control. Matching was done on the basis of age at screening, date of enrollment, race/ethnicity (white, black, Hispanic, other), and hysterectomy status at baseline. The final sample size included 1224 matched pairs, plus 10% blind duplicates for assay quality control. The WHI protocol was approved by the institutional review boards at the Clinical Coordinating Center at the Fred Hutchinson Cancer Research Center and the 40 clinical centers. Separate approval to use deidentified samples and data for the analyses proposed in this study was obtained from the Tufts University/Tufts Medical Center Institutional Review Board.

Plasma PL Fatty Acid Profiles and Desaturase Indexes

Plasma PL fatty acid profiles were determined by using an established gas chromatography method.[12] Peaks of interest were identified by comparison with authentic fatty acid standards (Nu-Check-Prep) and expressed as molar percentage (mol %) proportions of fatty acids relative to the internal standard. The mean±SD percent recovery of the internal standard was 93±3%. Blind duplicate analysis was performed on 10% of the samples, and the intra-assay coefficient of variation was <5% for 16:0, 18:0, and 18:2n-6; <10% for 15:0, 18:1n-9, 18:1t, 20:3n-6, 20:4n-6, 22:4n-6, 22:5n-6, 18:3n-3,

20:5n-3, 22:5n-3, and 22:6n-3; and 10% to 15% for 12:0, 14:0, 20:0, 22:0, 24:0, 16:1n-9, 16:1n-7, 18:1n-7, 20:1n-9, 24:1n-9, 18:3n-6, 20:2n-6, and 18:2t. Pooled plasma samples were also used as additional external quality controls and run with every 4 batches of samples. Instrument precision was determined by analyzing 10 serial injections from each of 3 control samples.

Plasma desaturase enzyme activities were estimated as product-to-precursor ratios of individual fatty acids and included stearoyl-CoA-desaturase (SCD-16; 16:1n-7/16:0 and SCD-18; 18:1n-9/18:0),[49–52] Δ6-desaturase (D6D; 20:3n-6/18:2n-6),[23,31,53,54] and Δ5-desaturase (D5D; 20:4n-6/20:3n-6).[54] An alternate way to estimate D6D activity, the ratio of 18:3n-6/18:2n-6, was not used due to the low proportion of 18:3n-6 in plasma PL.[23,31]

Covariates

Sociodemographic variables (age, race/ethnicity, income, marital status, education) were measured by interview or self-report at baseline using standardized questionnaires.[47] Traditional CHD risk factors were measured by self-report using questionnaires (smoking status, family history of MI, and frequency, intensity, and duration of physical activity) as well as by trained, certified staff at the baseline examination. Height was measured using a stadiometer, weight was measured with participants wearing light clothing, and body mass index was calculated (kg/m^2).

Statistical Analyses

Baseline characteristics were summarized for cases and controls using mean and SD values for normally distributed continuous variables and frequencies and percentages for categorical variables. Clinical characteristics, plasma PL fatty acid profiles, and desaturase indexes between cases and controls were compared using either paired *t*-test, Wilcoxon signed rank, or McNemar tests, depending on distribution of the data. Multivariate conditional logistic regression was used to obtain odds ratios (ORs) and corresponding 95% CIs for CHD and fatal/nonfatal MI risk for plasma PL fatty acids and OR with 95% CI per SD increase for desaturase activities. Covariate selection was based on previously documented CHD risk factors in postmenopausal women and/or those factors associated with exposure and outcomes in this study. In keeping with the principle of parsimony,[28] the following multivariate models were fitted. Model 1 controlled for matching factors (age, enrollment date, race/ethnicity, and hysterectomy status). Model 2 additionally controlled for body mass index, systolic blood pressure, smoking, education, medication (anticoagulant, antidiabetic, antilipid) and hormone use (estrogen and/or progesterone), family history of CVD/stroke/MI and type 2 diabetes, and leisure physical activity. Additionally, because the fatty acids in the DNL pathway are affected by carbohydrate, protein, and alcohol intake,[20,24] model 3 was adjusted for these variables (percent energy). In all models, we used plasma PL fatty acids as a continuous variable. Although multiple comparisons have been performed, we have not used a Bonferroni correction factor. This decision was made because *P* value adjustments, while reducing the chance of making a type I error, increase the chance of making a type II error. To mitigate these effects, both individual *P* values and CIs have been reported. Potential effect modifiers were investigated by assessing the significance of multiplicative interaction terms using Wald tests. None were statistically significant or improved the model(s) and, hence, only main effect terms were included. Analyses were performed using Stata 11 (StataCorp).

Results

The clinical characteristics of the cases and controls are depicted in Table 1. Mean age was 67.8±0.2 years. Cases had significantly higher body mass index and systolic and diastolic blood pressures and lower levels of education than control subjects. There was a higher proportion of smokers, medication/hormone use, and history of diabetes and stroke among cases, who also reported being less physically active.

Plasma PL Fatty Acid Profiles and Desaturase Indexes Between Cases and Controls

In plasma, the following fatty acids in the PL fraction were more abundant among cases than among controls: total saturated fatty acids (SFA), predominantly 18:0, and polyunsaturated ω6 fatty acids (PUFA-ω6), 18:3n-6, 20:3n-6, 22:4n-6, and 22:5n-6 (Table 2). The monounsaturated fatty acids (MUFA), 16:1n-9, 18:1n-7, and 20:1n-9, and PUFA ω3 fatty acids, 18:3n-3, 20:5n-3, 22:5n-3, and 22:6n-6, were significantly lower in the cases compared with the controls. No significant differences were observed in the proportion of other SFA (including 15:0), the PUFA ω6 (18:2n-6, 20:2n-6, 20:4n-6), or *trans*–fatty acids (total, 18:1t, 18:2t) between cases and controls.

The SCD-16 activity index (Table 3) was similar between cases and controls. A trend (*P*=0.06) toward lower SCD-18 activity in the cases relative to the controls was observed, suggesting less conversion of 18:0 to 18:1. The D6D index was significantly higher while the D5D index was significantly lower in the cases than in the controls, indicating higher

Table 1. Demographic and Clinical Characteristics of WHI-OS Controls and Cases

Variables	Controls (n=1224)	Cases (n=1224)	*P* Value*
Age, y†	67.8±0.2	67.8±0.2	Matched
Ethnicity (% white)	89.3	89.3	Matched
Body mass index, kg/m†	27.0±0.2	28.1±0.2	<0.01
Systolic blood pressure, mm Hg†	128.5±0.5	135.8±0.6	<0.01
Diastolic blood pressure, mm Hg†	73.9±0.3	75.7±0.3	<0.01
Education (highest level, %)			<0.01
Some high school or lower	4.1	6.5	
Complete high school	16.6	20.2	
Vocational or training school	10.6	11.7	
Some college/associated degree	26.6	26.1	
College graduate/baccalaureate degree	12.2	9.8	
Some postgraduate/professional degree	12.3	11.7	
Master's degree	14.7	11.9	
Doctoral degree (PhD, MD, JD)	2.9	2.2	
Smoking, %			<0.01
Never	53.6	47.9	
Past	41.2	43.9	
Current	5.2	8.2	
Medication/hormone use, % yes			
Anticoagulant medication	1.1	2.4	0.04
Diabetes medication	1.9	8.8	<0.01
Antihyperlipidemic medication	7.9	10.5	0.02
Estrogen or progesterone	24.3	20.1	<0.01
Family history, % yes			
Type 2 diabetes	36.2	31.6	<0.01
Stroke	38.8	42.1	0.12
Leisure physical activity, MET-h/wk†	14.5±0.4	11.8±0.4	<0.01

WHI-OS indicates Women's Health Initiative observational study; MET, metabolic equivalents.
**P* value derived using paired *t* test for continuous and normally distributed variables and the Wilcoxon signed rank and McNemar tests for continuous non–normally distributed and categorical data.
†Values are mean±standard error.

conversion of 18:2n-6 to 20:3n-6 but subsequently lower conversion of 20:3n-6 to 20:4n-6.

Plasma PL Fatty Acid Profiles and Desaturase Indexes as Related to CHD Risk

To further describe the relationship between dietary fat quality and CHD risk, we calculated the ORs (95% CIs) of CHD risk for plasma PL fatty acid profiles (Table 4, with model 3 results depicted in Figure). In all models, positive associations with odds of CHD were observed for PL total SFA (OR=1.120 [1.08 to 1.32]) but none of the individual SFA and CHD risk. PL-18:0 was associated with increased CHD risk in the initial model (OR=3.18 [1.47 to 6.85]), but this association was attenuated on adjustment for covariates (models 2 and 3). Among the PUFA ω6 fatty acids, in the initial model, 18:3n-6, 20:3n-6, 22:4n-6, and 22:5n-6 were associated with higher CHD risk but, after adjustment for all covariates, only 20:3n-6 (OR=3.22 [1.95 to 5.32]) and 22:5n-6 (OR=1.63 [1.19 to 2.23]) remained significant. No significant association was observed between 20:4n-6 and CHD risk (OR=0.68 [0.38 to 1.23]). Among the PUFA ω3 fatty acids, 20:5n-3 (OR=0.73 [0.58 to 0.93]), 22:5n-3 (OR=0.56 [0.33 to 0.94]), and 22:6n-3 (OR=0.56 [0.39 to 0.80, with the exception of 18:3n-3 (OR=0.77 [0.57 to 1.05]), were associated with lower CHD risk, with risk

Table 2. Plasma PL Fatty Acid Profiles (mol %) in WHI-OS Controls and Cases

Variables*	Controls (n=1224)	Cases (n=1224)	*P* Value†
SFA	45.98 ± 0.04	46.20 ± 0.04	<0.01
12:0, lauric	0.07 ± 0.001	0.07 ± 0.001	0.19
14:0, myristic	0.69 ± 0.01	0.69 ± 0.01	0.76
15:0, pentadecanoic	0.23 ± 0.001	0.23 ± 0.001	0.10
16:0, palmitic	30.54 ± 0.06	30.60 ± 0.06	0.44
18:0, stearic	13.11 ± 0.04	13.27 ± 0.04	<0.01
20:0, arachidic	0.24 ± 0.002	0.24 ± 0.002	0.27
22:0, behenic	0.64 ± 0.01	0.65 ± 0.01	0.37
24:0, tetracosanoic	0.46 ± 0.01	0.46 ± 0.01	0.56
MUFA	12.36 ± 0.05	12.27 ± 0.05	0.13
16:1n-9, palmitoleic	0.12 ± 0.001	0.11 ± 0.001	0.02
16:1n-7, hexadecenoic	0.83 ± 0.01	0.84 ± 0.01	0.79
18:1n-9, oleic	8.50 ± 0.04	8.46 ± 0.04	0.40
18:1n-7, *cis*-vaccenic	1.40 ± 0.01	1.35 ± 0.01	<0.01
20:1n-9, eicosanoic	0.08 ± 0.001	0.07 ± 0.001	< 0.01
24:1n-9, tetracosenoic	0.78 ± 0.01	0.77 ± 0.01	0.20
PUFA n-6	36.23 ± 0.07	36.38 ± 0.07	0.11
18:2n-6, linoleic	20.77 ± 0.08	20.82 ± 0.09	0.73
18:3n-6, γ-linoleic	0.09 ± 0.001	0.10 ± 0.001	<0.01
20:2n-6, eicosadienoic	0.40 ± 0.01	0.40 ± 0.01	0.48
20:3n-6, eicosatrienoic	3.25 ± 0.02	3.39 ± 0.03	<0.01
20:4n-6, arachidonic	10.97 ± 0.06	10.89 ± 0.06	0.30
22:4n-6, docosatetranoic	0.41 ± 0.003	0.42 ± 0.003	<0.01
22:5n-6, decosapentanoic	0.34 ± 0.004	0.36 ± 0.004	<0.01
PUFA n-3	5.28 ± 0.05	5.01 ± 0.04	<0.01
18:3n-3, α-linolenic	0.21 ± 0.002	0.20 ± 0.002	0.01
20:5n-3, eicosapentanoic	0.84 ± 0.01	0.78 ± 0.01	0.01
22:5n-3, docosapentanoic	0.84 ± 0.01	0.83 ± 0.01	0.05
22:6n-3, decosahexanoic	3.37 ± 0.03	3.19 ± 0.03	0.01
Total *trans*	0.68 ± 0.01	0.69 ± 0.01	0.65
18:1, all 18:1 *trans*	0.54 ± 0.01	0.54 ± 0.01	0.69
18:2t, all 18:2 *trans*	0.14 ± 0.002	0.14 ± 0.002	0.18

WHI-OS indicates Women's Health Initiative observational study; PL, phospholipid; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.
*Values are mean ± standard error.
†*P* value derived using paired *t* test.

reductions ranging from 27% to 44% in the fully adjusted model. Adding the covariates, fish, vegetables, and fruits (servings/day) to model 3 resulted in similar results.

Limited evidence suggests that fatty acids in the DNL pathway may be associated with increased CHD risk. In our cohort, after additional adjustment for percent of energy from carbohydrate, protein, and alcohol in model 3, MUFA 18:1n-7 (OR=0.54 [0.29 to 0.99]) was associated with a 46% lower risk of CHD. No significant associations were detected for 16:1n-7 (OR=1.03 [0.76 to 1.47]), 16:1n-9 (OR=0.84 [0.58 to 1.22]), or SFA 16:0 (OR=3.26 [0.51 to 20.81]) or 18:0 (OR=1.57 [0.53 to 4.61]).

With regard to desaturase activity indexes, neither SCD-16 (OR=1.01 [0.99 to 1.01]) nor SCD-18 (OR=0.93 [0.83 to 1.05]) was associated with risk of CHD (values are OR with 95% CI for CHD per SD increase of log-transformed desaturase index). The

Table 3. Estimated Fatty Acid Desaturase Activity Indexes in WHI-OS Controls and Cases

Desaturase Index*	Controls (n=1224)	Cases (n=1224)	*P* Value†
Stearoyl-CoA-desaturase (SCD)			
SCD-16; 16:1n-7/16:0	0.03±0.0003	0.03±0.0003	0.64
SCD-18; 18:1n-9/18:0	0.66±0.004	0.65±0.004	0.06
Δ6-Desaturase			
20:3n-6/18:2n-6	0.16±0.001	0.17±0.001	<0.001
Δ5-Desaturase			
20:4n-6/20:3n-6	3.60±0.034	3.41±0.032	<0.001

WHI-OS indicates Women's Health Initiative observational study.
*Values are mean±standard error.
†*P* value derived using paired *t* test.

D6D index was associated with a higher risk of CHD (OR=1.25 [1.11 to 1.41]), while the D5D index was associated with lower CHD risk (OR=0.78 [0.70 to 0.88]).

Discussion

This prospective nested case-control study assessed the impact of plasma PL fatty acids profiles, which reflect both dietary intake and in vivo fatty acid metabolism, on CHD risk, in a cohort of free-living postmenopausal women. Most strongly associated with CHD risk were the very long–chain ω3 fatty acids. While, no significant associations were observed for the predominant PL fatty acids (16:0, 18:0, 18:1n-9, and 18:2n-6) and CHD risk, significant positive associations were found between plasma PL total SFA and the PUFA ω6 fatty acids 20:3n-6 and 22:5n-6 with CHD risk. An inverse association was observed between 18:1n-7, a fatty acid in the DNL pathway, and CHD risk. These associations were partly explained by changes in estimated desaturase activity, demonstrated by significantly higher D6D and lower D5D indexes.

A modest positive association between total SFA but not individual even-chain SFA (14:0, 16:0, 18:0, 22:0, and 24:0) and CHD risk was observed in our study. These results are consistent with 4[25,28,37,55] of the 7 cohort studies that have assessed either total or individual SFA in the PL[39,45] or CE fraction.[31] This association most likely reflects endogenous DNL because the SFA and the MUFA content of blood lipid fractions are weaker biomarkers than PUFA or *trans*–fatty acids for their respective dietary intakes.

PL15:0, an odd-chain SFA, derived from ruminant sources and considered to be a biomarker of dairy intake,[55] was not related to CHD risk in our study. These findings are consistent with some[21,45] but not all cohort studies.[20,39,55–57] Of note, the PL-15:0 concentration in our cohort was similar to those reported previously,[55,57] so it is unlikely that our group of women were not high consumers of full-fat dairy products. However, it is possible that they may have intentionally chosen low and reduced fat dairy products, consistent with current public health messaging.

Total *trans*–fatty acids, have been positively associated with CHD risk.[58] However, total *trans*–fatty acids represent a mixture of *trans*–double bond–containing isomers, the relative proportion of which differs between the 2 major dietary sources: partially-hydrogenated fat and animal fat. It has been suggested that the predominant *trans*–fatty acid in dairy products, vaccenic acid (18:1n-11t), may have a weaker association with CHD risk than *trans*–fatty acids resulting from partial hydrogenation of vegetable oils, mainly elaidic acid (18:1n-9t).[59] Our GC methodology at the time did not allow for the separation of *trans*–fatty acid isomers. Consequently our PL 18:1t data included both vaccenic and elaidic acids, which could have potentially attenuated the association with CHD.

In our cohort, no association was observed between 18:1n-9, the predominant dietary MUFA, as well as 16:1n-9, 16:1n-7, 20:1n-9, and 24:1n-9 and CHD risk, a finding similar to that reported in other cohorts.[25,37–39,45] However, 18:1n-7, a product of the DNL pathway, was associated with lower CHD risk. We reported a similar inverse association between red blood cell 18:1n-7 and CHD risk (OR=0.79 [0.69 to 0.91]) in the Physicians Health Study.[60] This finding is in contrast to that reported in the European Prospective Investigation into Cancer (EPIC)-Norfolk cohort where PL 18:1n-7 was associated with higher risk of CHD (OR=1.17 [1.06 to 1.31]).[39] Interestingly, in the Cardiovascular Health Study, PL 18:1n-7 was associated with higher risk of sudden cardiac death (OR=7.63 [2.58 to 22.6]) but not total CHD.[28] The underlying mechanisms by which 18:1n-7 might influence the risk of CHD have not yet been elucidated.

Among the plasma PL fatty acid biomarkers of dietary fish intake, our estimated associations between 20:5n-3, 22:5n-3, and 22:6n-3 and CHD risk are broadly consistent with the majority of studies that measured ω3 fatty acids in either PL,[14,15,37,61] or other lipid fractions,[62] as well as erythrocytes/whole blood[63,64] and plasma/serum.[25,62,63] In contrast, 18:3n-3, a plant-derived omega 3, was not associated with CHD risk in our cohort. Of note, a recent systematic review and meta-analysis of 18:3n-3 and risk of CVD found that dietary 18:3n-3 intake, assessed using food frequency questionnaires, diet histories, or diet records, but not 18:3n-3 biomarker concentrations, measured in blood or adipose tissue, was associated with a modestly lower risk of CVD,[65,66] further highlighting the differential effect of diet-derived fatty acid intake versus circulating fatty acid concentrations on CVD outcomes.

The predominant dietary PUFA omega 6, 18:2n-6, was not associated with CHD risk. This is similar to that reported in

Table 4. Association Between Plasma PL Fatty Acids and CHD Risk in WHI-OS Participants (N=2448)

Variables	Odds Ratio (95% CI)*		
	Model 1[†]	Model 2[‡]	Model 3[§]
SFA	1.19 (1.11 to 1.28)[‖]	1.19 (1.07 to 1.31)[‖]	1.20 (1.08 to 1.32)[‖]
12:0, lauric	0.85 (0.67 to 1.08)	0.95 (0.69 to 1.31)	0.93 (0.68 to 1.28)
14:0, myristic	0.95 (0.70 to 1.29)	1.06 (0.71 to 1.58)	1.05 (0.70 to 1.56)
15:0, pentadecanoic	0.73 (0.50 to 1.06)	0.97 (0.60 to 1.58)	1.02 (0.61 to 1.72)
16:0, palmitic	1.68 (0.45 to 6.25)	2.65 (0.45 to 15.70)	3.26 (0.51 to 20.81)
18:0, stearic	3.18 (1.47 to 6.85)[‖]	1.55 (0.54 to 4.43)	1.57 (0.53 to 4.61)
20:0, arachidic	0.81 (0.56 to 1.17)	1.01 (0.62 to 1.65)	0.99 (0.60 to 1.62)
22:0, behenic	1.14 (0.85 to 1.53)	1.40 (0.95 to 2.07)	1.35 (0.90 to 2.01)
24:0, tetracosanoic	0.92 (0.69 to 1.22)	1.28 (0.87 to 1.87)	1.22 (0.83 to 1.80)
MUFA	0.96 (0.91 to 1.01)	0.96 (0.90 to 1.03)	0.97 (0.91 to 1.04)
16:1n-7, hexadecenoic	1.01 (0.81 to 1.32)	1.00 (0.73 to 1.38)	1.06 (0.76 to 1.47)
16:1n-9, palmitoleic	0.71 (0.53 to 0.95)[‖]	0.80 (0.55 to 1.15)	0.84 (0.58 to 1.22)
18:1n-7, *cis* vaccenic	0.33 (0.21 to 0.53)[‖]	0.48 (0.26 to 0.88)[‖]	0.54 (0.29 to 0.99)[‖]
18:1n-9, oleic	0.79 (0.46 to 1.36)	0.69 (0.34 to 1.42)	0.71 (0.34 to 1.46)
20:1n-9, eicosanoic	0.72 (0.57 to 0.91)[‖]	0.83 (0.61 to 1.14)	0.85 (0.62 to 1.17)
24:1n-9, tetracosenoic	0.84 (0.64 to 1.10)	1.10 (0.77 to 1.57)	1.12 (0.78 to 1.61)
PUFA n-6	1.03 (0.99 to 1.06)	1.03 (0.98 to 1.08)	1.02 (0.97 to 1.07)
18:2n-6, linoleic	1.10 (0.63 to 1.92)	1.19 (0.56 to 2.52)	0.94 (0.43 to 2.07)
18:3n-6, γ-linoleic	1.29 (1.09 to 1.54)[‖]	1.22 (0.97 to 1.54)	1.24 (0.98 to 1.56)
20:2n-6, eicosadienoic	0.92 (0.72 to 1.17)	0.92 (0.67 to 1.27)	0.96 (0.69 to 1.32)
20:3n-6, eicosatrienoic	2.36 (1.65 to 3.39)[‖]	2.93 (1.79 to 4.80)[‖]	3.22 (1.95 to 5.32)[‖]
20:4n-6, arachidonic	0.80 (0.52 to 1.22)	0.68 (0.37 to 1.18)	0.68 (0.38 to 1.23)
22:4n-6, docosatetranoic	1.63 (1.22 to 2.18)[‖]	1.30 (0.89 to 1.90)	1.39 (0.94 to 2.06)
22:5n-6, decosapentanoic	1.52 (1.22 to 1.89)[‖]	1.56 (1.15 to 2.11)[‖]	1.63 (1.20 to 2.23)[‖]
PUFA n-3	0.88 (0.84 to 0.93)[‖]	0.89 (0.83 to 0.96)[‖]	0.89 (0.83 to 0.97)[‖]
18:3n-3, α-linolenic	0.72 (0.57 to 0.90)[‖]	0.77 (0.57 to 1.04)	0.77 (0.57 to 1.05)
20:5n-3, eicosapentanoic	0.74 (0.63 to 0.89)[‖]	0.73 (0.58 to 0.92)[‖]	0.73 (0.58 to 0.93)[‖]
22:5n-3, docosapentanoic	0.71 (0.49 to 1.01)	0.51 (0.31 to 0.56)[‖]	0.56 (0.33 to 0.94)[‖]
22:6n-3, decosahexanoic	0.54 (0.42 to 0.70)[‖]	0.55 (0.39 to 0.77)[‖]	0.56 (0.39 to 0.80)[‖]
Total *trans*	1.01 (0.89 to 1.22)	1.04 (0.81 to 1.36)	1.00 (0.81 to 1.24)
18:1, all 18:1 *trans*	1.03 (0.89 to 1.18)	0.99 (0.82 to 1.19)	0.99 (0.82 to 1.20)
18:2t, all 18:2 *trans*	1.14 (0.94 to 1.39)	1.05 (0.81 to 1.36)	1.05 (0.81 to 1.37)

PL indicates phospholipid; CHD, coronary heart disease; WHI-OS, Women's Health Initiative observational study; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

*Conditional logistic regression was used to obtain odds ratios and corresponding 95% CIs for CHD and fatal/nonfatal MI risk associated with increasing concentrations of each individual or groups of plasma PL fatty acids.

[†]Model 1: age, enrollment date, race/ethnicity, and hysterectomy status.

[‡]Model 2: model 1 covariates plus body mass index, systolic blood pressure, smoking, education, medication (anticoagulant, antidiabetic, antilipid) and hormone (estrogen and/or progesterone) use, family history of cardiovascular disease/stroke/MI and type 2 diabetes, and leisure physical activity.

[§]Model 3: model 2 covariates plus carbohydrate, protein, and alcohol intake (percent energy).

[‖]Statistically significant.

the Atherosclerosis Risk In Communities (ARIC), Multiple Risk Factor Intervention Trial (MRFIT), and Whitehall study cohorts[25,37,45] but not the EPIC-Norfolk cohort,[39] which reported a significantly lower risk of CHD (OR=0.66 [0.58 to 0.75]) with 18:2n-6. This most likely indicates higher dietary ω6 intake in this cohort as reflected by higher PL ω6

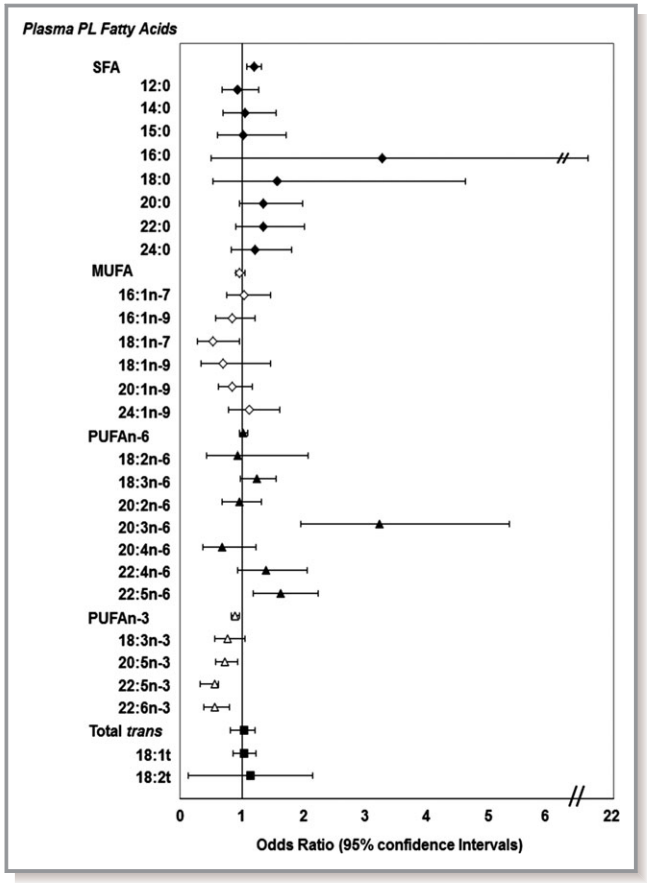

Figure. Association between plasma PL fatty acids and CHD risk in WHI-OS participants. Multivariate conditional logistic regression was used to obtain odds ratios and corresponding 95% CIs for CHD and fatal/nonfatal MI associated with increasing concentrations of plasma nutrient biomarker concentrations, after controlling for BMI, systolic blood pressure, smoking, education, medication/hormone use, family history of CVD/diabetes, and leisure physical activity. Error bars represent 95% CIs. BMI indicates body mass index; CHD, coronary heart disease; CVD, cardiovascular disease; MI, myocardial infarction; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; WHI-OS, Women's Health Initiative observational study.

concentrations (40 mol% versus 35 mol% in the present study). Interestingly, in our cohort, endogenously synthesized 20:3n-6 and 22:5n-6, but not 20:4n-6, was associated with a higher risk of CHD. This lack of association between 20:4n-6 and CHD risk has been observed in other prospective cohort studies.[25,37–39,45] Because dietary fat quality influences the activity of enzymes involved in the desaturation of fatty acids,[67] these results could reflect activity of these enzymes. Among the desaturase enzyme activities estimated in our study, SCD-16 and SCD-18, also referred to as Δ9-desaturase, which catalyzes the conversion of SFA to MUFA, was not associated with CHD risk in our cohort. With regard to the estimated ω6 desaturase enzyme activities, we found that D6D, which desaturates 18:2n-6 to 18:3n-6 and subsequently 20:3n-6, was associated with higher CHD risk. In contrast, D5D activity, reflecting conversion of 20:3n-6 to 20:4n-6, a substrate for the series 2 thromboxanes and eicosanoids, proinflammatory cytokines, was associated with lower CHD risk. These data are consistent with those reported for D6D estimated in the PL or CE fraction and heart failure/CVD mortality.[31,62] Similarly, 4 studies have reported an inverse association between D5D activity estimated using the CE fraction and MI,[38] heart failure,[62] as well as with incident CHD.[31,68] We hypothesize that these data reflect lower production of 20:4n-6 from its precursor, 20:3n-6, and/or further elongation and desaturation to other long-chain ω6 fatty acids, 22:4n-6 and 22:5n-6.

Our study had several strengths as well as limitations. Given the observational nature of the study design, we cannot exclude chance or residual/unmeasured confounding as an alternative explanation of the study results. However, the use of matching and adjustment of several potential covariates to minimize confounding and the standardized methods for follow-up and identification of cases in the WHI-OS cohort were strengths of the present study. While case-control study designs are bias prone, by using a nested case-control design, we leveraged a prospective cohort study to select cases before the diagnosis and used typical risk set sampling to select controls, thus reducing the bias and uncertainty regarding the temporal sequence between exposure and disease onset. All study participants were postmenopausal women, which limits the generalizability of our findings but provides valuable information for the development of evidence-based guidelines for this high-risk and growing subgroup of the population. We were limited by our use of a single fasting baseline blood sample for the biomarker analyses. While measurements at multiple time points may have better reflected long-term intake, multiple samples do not always add benefit to validation analysis.[69] Finally, estimated associations between desaturase activities and CHD risk offers indirect, rather than direct, measures of these enzyme activities.[67]

In conclusion, plasma PL fatty acid concentrations, specifically total SFA, MUFA 18:1n-7, PUFA ω6 (20:3n-6, 22:5n-6), and ω3 (20:5n-3, 22:5n-3, 22:6n-6), were predictive of CHD risk, positively and negatively, respectively, among postmenopausal women participating in the WHI-OS. These results confirm the cardioprotective effect of very long–chain ω3 fatty acids and support current recommendations for regular fish consumption. An additional finding is that indicators of endogenous fatty acid metabolism may also play an important role in predicting CHD risk.

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Disclosures

None.

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