

Plasma Phospholipid Saturated Fatty Acids and Incident Atrial Fibrillation: The Cardiovascular Health Study

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Background—Prior studies suggest that circulating fatty acids may influence the risk of atrial fibrillation (AF), but little is known about the associations of circulating saturated fatty acids with risk of AF.

Methods and Results—The study population included 2899 participants from the Cardiovascular Health Study, a community-based longitudinal cohort of adults aged 65 years or older in the United States who were free of prevalent coronary heart disease and AF in 1992. Cox regression was used to assess the association of all the long-chain saturated fatty acids—palmitic acid (16:0), stearic acid (18:0), arachidic acid (20:0), behenic acid (22:0), and lignoceric acid (24:0)—with incident AF. During a median of 11.2 years of follow-up, 707 cases of incident AF occurred. After adjustment for other AF risk factors, higher levels of circulating 16:0 were associated with a higher risk of AF (hazard ratio comparing highest and lowest quartiles: 1.48; 95% CI: 1.18, 1.86). In contrast, higher levels of circulating 18:0, 20:0, 22:0, and 24:0 were each associated with a lower risk of AF. The hazard ratios (95% CI) for AF in the top and bottom quartiles were 0.76 (95% CI: 0.61, 0.95) for 18:0; 0.78 (95% CI: 0.63, 0.97) for 20:0; 0.62 (95% CI: 0.50, 0.78) for 22:0; and 0.68 (95% CI: 0.55, 0.85) for 24:0.

Conclusions—Results from this prospective cohort study of older adults demonstrate divergent associations of circulating 16:0 versus longer-chain saturated fatty acids with incident AF, highlighting the need to investigate both determinants of these levels and potential pathways of the observed differential risk. (*J Am Heart Assoc.* 2014;3:e000889 doi: 10.1161/JAHA.114.000889)

Key Words: atrial fibrillation • epidemiology • fatty acid

Atrial fibrillation (AF) is a common chronic arrhythmia among older adults. In the United States, more than 3 million individuals have diagnosed AF, and this estimate is

expected to reach more than 7.5 million by 2050, as the population ages.¹ Because AF is associated with a higher risk of morbidity, including stroke, heart failure, cognitive decline, and dementia,^{2,3} identification of risk factors associated with AF is of considerable public health importance.

Prior studies suggest that circulating fatty acids may influence the risk of AF. In the Cardiovascular Health Study (CHS), plasma phospholipid long-chain n-3 fatty acids derived from seafood, particularly eicosapentaenoic acid and docosahexaenoic acid, were inversely associated with risk of AF.⁴ Conversely, there was no evidence of an association of circulating levels of plant-derived α -linolenic acid and AF.⁵ Little is known about the associations of circulating saturated fatty acids (SFAs) with AF. SFAs are derived from both endogenous and exogenous sources, and for this reason, circulating SFAs generally show low correlations with dietary intake of SFAs. SFAs of different length differ in incorporation into membrane lipid species, membrane properties, and biological activity. In addition, SFAs also differ in their dietary sources. The SFAs palmitic acid (16:0) and stearic acid (18:0) are found in animal products, such as meat and hard cheeses, and in tropical oils, whereas arachidic acid (20:0), behenic

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acid (22:0), and lignoceric acid (24:0) are found in peanuts and canola oil. SFA 16:0 is also the primary end product of fatty acid synthesis,^{6–8} and circulating levels of 16:0 are influenced by carbohydrate and alcohol intake.^{9–11}

To date, some observational studies,^{12–16} but not all,^{11,17} suggest that individual SFAs may have differential effects of risk on cardiovascular disease and its risk factors. A handful of *in vitro* experiments with cardiac myocytes did not find any effect of 18:0 on calcium and sodium currents or membrane electrical excitability,^{18–20} and no published studies have examined the effect of very long-chain SFAs 20:0, 22:0, and 24:0 on electrophysiology parameters. To elucidate how levels of different circulating SFAs are associated with AF, we examined the associations of plasma phospholipid 16:0, 18:0, 20:0, 22:0, and 24:0 with incident AF in the CHS, a population-based longitudinal cohort of older adults.

Methods

Study Population

The CHS is a longitudinal study of cardiovascular disease among noninstitutionalized adults aged 65 years or older from 4 communities in the United States (Forsyth County, North Carolina; Sacramento County, California; Washington County, Maryland; Allegheny County, Pennsylvania). Details of the study design and sampling procedures have been reported previously.²¹ In total, 5201 participants enrolled in the study in 1989–1990 and 687 participants (largely black) enrolled in 1992–1993. Study participants were followed by annual clinic visits with interim 6-month follow-up phone calls until 1999–2000 and by phone contact every 6 months thereafter. The institutional review board at each site approved the study, and written informed consent was obtained from participants at enrollment.

Plasma phospholipid fatty acids were measured in 3941 participants using stored blood samples from 1992 to 1993, considered the baseline for the present analysis. We excluded 233 participants with prevalent AF and 809 participants with prevalent coronary heart disease because a diagnosis of coronary heart disease may have influenced diet and plasma phospholipid fatty acid levels. The remaining 2899 persons composed the study population for the current analysis.

Data Collection

At baseline and during follow-up, participants underwent comprehensive annual clinic examinations that included a standardized interview, physical examination, laboratory assessment, and diagnostic tests. Information regarding past medical conditions, smoking, alcohol consumption, and other health behaviors were collected. Blood samples were collected

after a 12-hour overnight fast and stored at -70°C . To measure usual dietary intake, reproducible and validated food frequency questionnaires (FFQs) were administered in 1989 and 1996. The 1989 FFQ was a picture version of the National Cancer Registry FFQ and included 99 food items.²² In 1996, the Willett 131-item semiquantitative FFQ was administered.²³ Because the blood samples used to measure plasma phospholipid fatty acids for the present analysis were collected during 1992–1993, we cumulatively updated responses to the 2 FFQs for participants who completed both the 1990 and the 1996 FFQs and did not develop coronary heart disease during 1989–1996 ($n=1927$). For participants who developed coronary heart disease during 1989–1996, only the 1989–1990 dietary measure was used for analyses ($n=202$). We chose not to update diet among participants who developed coronary heart disease during follow-up because diagnosis may have influenced diet and risk of AF. For participants who enrolled in CHS during 1992–1993 ($n=263$), the 1995–1996 diet measure was used for analyses.

Assessment of Plasma Phospholipid SFAs

Plasma phospholipid fatty acids were measured at the Fred Hutchinson Cancer Research Center (Seattle, Washington) using stored blood samples from 1992 to 1993. Total lipids were extracted from plasma using the methods of Folch et al.²⁴ One-dimensional thin-layer chromatography was used to separate phospholipids from neutral lipids. Phospholipids fraction was directly transesterified using the method of Lepage and Roy to prepare fatty acid methyl esters,²⁵ and individual fatty acid methyl esters were separated using gas chromatography (Agilent 5890 Gas Chromatograph flame ionization detector, Agilent Technologies; fused silica capillary column SP-2560 [100×0.25 mm, 0.2 μm], Supelco; initial 160°C for 16 minutes, ramp 3°C per minute to 240°C, hold 15 minutes).²⁶ All fatty acids were processed at the biomarker laboratory of the Fred Hutchinson Cancer Research Center. For this analysis, levels of each individual fatty acid including 16:0, 18:0, 20:0, 22:0, and 24:0 are expressed as a weight percentage of total phospholipid fatty acids analyzed.

Ascertainment of AF

Incident AF, including atrial flutter, was identified from 12-lead ECGs performed annually until 1999 or was based on hospital discharge diagnoses (International Classification of Diseases, 9th revision codes 427.3, 427.31, 427.32) through June 30, 2008. Review of medical records of a subsample of participants in the CHS with a hospital discharge code for AF indicates that the positive predictive value of AF identification through hospital diagnosis codes was 98.6%.²⁷ In addition, among 819 CHS participants who underwent a 24-hour Holter

monitor assessment at the 1994–1995 examination, only 1 study participant had sustained or intermittent AF identified by the Holter monitor that was not identified by either ECG or hospital discharge diagnosis codes.²⁸

Statistical Analyses

All statistical analyses were conducted using Stata version 10.0 (StataCorp). Cox proportional hazard models were used to examine the associations of each plasma phospholipid SFA with incident AF. Plasma phospholipid SFAs were assessed categorically using indicator quartiles. We examined the associations of individual SFAs with the hazard of developing AF in live subjects, so deaths from any cause were treated the same way as censoring due to loss to follow-up (1211 participants censored due to death). All participants were followed until the end of follow-up (June 30, 2008), loss to follow-up, date of death, or development of AF. We did not run formal analyses to assess the competing risk of death because our focus was solely on AF risk. The proportional hazards assumption for each SFA was evaluated using Schoenfeld's residuals.

Two levels of adjustment were used to examine the associations of each SFA of interest with incident AF. Minimal adjustments included age and sex. The primary model additionally included race (European American, African American, other), enrollment site (Forsyth County, North Carolina; Sacramento County, California; Washington County, Maryland; Allegheny County, Pennsylvania), education (no high school, high school or vocational school, college), and body mass index (kilograms per square meter), waist circumference (centimeters), smoking (never, past, current), alcohol use (drinks per week), physical activity (kilocalories per week), diabetes (yes or no), history of heart failure (yes or no), history of stroke (yes or no), treated hypertension (yes or no), and plasma phospholipid long-chain n-3 fatty acids (percentage of total fatty acids) at baseline. Because exposure misclassification may increase with increasing duration of follow-up and ascertainment of AF may have been better in the earlier years

of follow-up (ie, AF ascertained by both ECG and hospital discharge code through 1999), we performed sensitivity analyses censoring at the midpoint of follow-up, 8 years after the 1992–1993 blood draw. It is possible that the associations between SFAs and AF may differ by sex, age, or hypertension status,^{29,30} and we examined potential effect modification of each of these factors with each SFA (modeled linearly) on risk of AF by evaluating the statistical significance of the multiplicative interaction term using Wald's tests. We also performed sensitivity analyses stratified by sex, age (\pm median, 74 years), and hypertension status (yes or no) as well as cross-tabulation analyses stratified at the median of each SFA of interest and age. Finally, because the SFAs of interest were correlated, we performed exploratory models that additionally adjusted for SFA 16:0 and for all other SFAs (ie, 16:0, 18:0, 20:0, 22:0, and 24:0, as appropriate).

Missing covariates (<2% for all covariates, except alcohol intake; missing 4.0%) were imputed by multiple imputations using data on age, sex, smoking, education, race, body mass index, physical activity, self-reported health status, and diabetes at the time of the plasma phospholipid SFA measure.

Results

At baseline in 1992–1993, the median age was 74 years (range: 65 to 98 years), and 63.6% of the sample was female. Levels of SFA were between 0.16% and 33.1% of total plasma phospholipid fatty acids (Table 1). The highest values were observed for 16:0 (mean \pm SD: 25.4 \pm 1.59), whereas the lowest values were observed for 20:0 (mean \pm SD: 0.50 \pm 0.08). Plasma phospholipid levels of 16:0, 18:0, 20:0, 22:0, and 24:0 were generally modestly intercorrelated, except for higher correlations of 20:0 and 22:0 ($r=0.63$) and 22:0 and 24:0 ($r=0.88$). Notably, all intercorrelations were positive except for those of 16:0, which inversely correlated with the other SFAs.

Characteristics of the study participants according to levels of plasma phospholipids 16:0 and 24:0 are shown in Table 2. In these crude (unadjusted) analyses, participants

Table 1. Pearson Correlation Coefficients for Plasma Phospholipid Saturated Fatty Acids

	16:0	18:0	20:0	22:0	24:0
Percentage of Total Fatty Acids*	25.4 (1.59)	13.4 (1.10)	0.50 (0.08)	1.68 (0.32)	1.40 (0.29)
16:0	1.00				
18:0	−0.52	1.00			
20:0	−0.31	0.20	1.00		
22:0	−0.46	0.15	0.63	1.00	
24:0	−0.36	0.07	0.46	0.88	1.00

*Values are mean (SD).

Table 2. Baseline Characteristics of Study Participants in Total and According to Plasma Phospholipid 16:0 and 24:0*

	Total	Quartile of 16:0				Quartile of 24:0			
		Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
% Total FA, median	NA	23.7	24.8	25.7	27.2	1.1	1.3	1.5	1.7
Range	NA	19.5 to 24.3	24.3 to 25.3	25.3 to 26.3	26.3 to 33.1	0.52 to 1.2	1.2 to 1.4	1.4 to 1.6	1.6 to 3.4
N	2899	723	728	722	726	698	720	724	757
Age, y	74.7 (5.1)	74.5 (5.2)	74.4 (5.1)	75.3 (5.3)	74.7 (4.9)	75.4 (5.4)	75.1 (5.2)	74.6 (5.0)	74.0 (4.8)
Sex, % male	36.4%	27.1%	40.1%	41.4%	36.8%	30.5%	31.9%	41.0%	41.5%
Race, % white	86.7%	78.8%	86.7%	89.6%	91.6%	90.1%	88.6	88.5%	79.9%
Education, % >high school	37.4%	28.8%	36.4%	42.0%	42.6%	35.1%	32.2%	42.0%	40.2%
Current smoker, %	9.6	9.0	9.8	9.0	10.5	10.7	9.7	8.8	9.0
Alcohol, drinks/week	0 (0 to 1)	0 (0 to 0.12)	0 (0 to 1)	0 (0 to 1)	0 (0 to 6.75)	0 (0 to 0.75)	0 (0 to 1)	0.02 (0 to 1.25)	0 (0 to 1.25)
Body mass index, kg/m ²	26.8 (4.7)	26.0 (4.4)	26.8 (4.6)	27.4 (5.1)	26.9 (4.7)	27.5 (5.1)	26.9 (4.8)	26.5 (4.5)	26.3 (4.3)
Waist circumference, cm	97.0 (13.4)	94.7 (12.8)	96.9 (12.9)	98.7 (14.1)	97.8 (13.3)	99.3 (14.6)	97.3 (13.4)	96.3 (12.8)	95.4 (12.3)
Diabetes mellitus, %	14.5	9.5	14.0	14.4	20.1	20.8	12.2	11.2	14.1
Treated hypertension, %	36.5	36.9	36.1	34.7	38.2	45.1	36.6	32.1	32.5
Aspirin >2 days in 2 weeks, %	30.4	32.2	27.9	31.0	30.7	28.6	31.0	30.5	31.5
Lipid-lowering medication, %	5.7	6.8	5.8	4.3	5.8	8.0	5.7	5.1	4.0
Hx of stroke, %	3.8	3.3	3.6	4.4	4.0	4.9	4.0	3.5	3.0
Hx of heart failure, %	2.2	2.2	1.8	2.8	1.9	3.4	1.8	1.7	1.8
Cystatin C, mg/L	1.1 (0.3)	1.1 (0.3)	1.1 (0.3)	1.1 (0.3)	1.1 (0.3)	1.2 (0.3)	1.1 (0.3)	1.1 (0.2)	1.1 (0.3)
eGFR <60 mL/min per 1.73 m ² , %	20.0	19.1	20.8	21.6	19.5	28.0	19.4	18.6	15.6
Carbohydrate, % energy	53.9 (7.2)	54.5 (7.2)	53.6 (6.6)	53.9 (7.0)	53.5 (7.9)	54.4 (7.7)	54.0 (7.1)	53.6 (7.0)	53.6 (7.1)
Total fat, % energy	30.8 (5.6)	30.9 (5.6)	31.4 (5.3)	30.8 (5.5)	30.3 (5.8)	30.3 (5.7)	30.8 (5.4)	31.0 (5.4)	31.2 (5.7)
Saturated fat, % energy	10.2 (2.2)	10.1 (2.2)	10.3 (2.1)	10.2 (2.3)	10.2 (2.3)	10.1 (2.3)	10.2 (2.2)	10.2 (2.1)	10.3 (2.3)
Polyunsaturated fat, % energy	6.6 (1.8)	6.6 (1.7)	6.7 (1.8)	6.5 (1.7)	6.4 (1.8)	6.4 (1.9)	6.5 (1.7)	6.7 (1.7)	6.7 (1.7)
Peanut intake, servings/week	0.46 (0.14 to 2.5)	0.46 (0.14 to 2.5)	0.46 (0.14 to 2.5)	0.46 (0.14 to 2.5)	0.46 (0.14 to 2.5)	0.46 (0.14 to 2.5)	0.46 (0.14 to 2.5)	0.46 (0.14 to 2.5)	0.46 (0.14 to 2.5)

eGFR indicates estimated glomerular filtration rate; FA, fatty acid; Hx, history.

*Data are mean (SD), percentage or median (interquartile range).

with higher 16:0 were more likely to be male and white, were more highly educated, consumed more alcohol per week, and were more likely to have diabetes when compared with participants with lower 16:0. Conversely, minimal differences according to 16:0 levels were evident in other AF risk factors, including age; smoking status; body mass index; aspirin or lipid-lowering drug use; cystatin C; estimated glomerular filtration rate; or prevalent treated hypertension, stroke, or heart failure. Similar to 16:0, participants with higher circulating 24:0 were more likely to be male and have higher education when compared with participants with lower circulating 24:0. In contrast to 16:0, participants with higher 24:0 were younger, less likely to be white, had slightly lower body mass index, and were less likely to have estimated glomerular filtration rate <60 mL/min per 1.73 m². Participants with higher levels of circulating 24:0 also had slightly higher dietary intake of peanuts, total fat, and polyunsaturated fat when compared with participants with lower levels of circulating 24:0. There were no apparent differences in alcohol intake; aspirin use; or histories of diabetes, hypertension, or heart failure, according to quartile of plasma phospholipid 24:0. Baseline characteristics of study participants according to quartiles of plasma phospholipid 18:0, 20:0, and 22:0 are provided in Table 3.

Plasma Phospholipid SFAs and Incident AF

During 29 864 person-years of follow-up, 707 cases of incident AF occurred. In both age- and sex-adjusted and multivariable analyses, higher levels of circulating 16:0 were associated with a higher risk of AF (Table 4). Risk estimates across quartiles suggested a monotonic dose response, with multivariate hazard ratios of 1.12 (95% CI: 0.89 to 1.40), 1.25 (95% CI: 0.99 to 1.56), and 1.48 (95% CI: 1.18 to 1.86) from the second to fourth quartiles compared with the first (P -trend <0.0001). Conversely, higher circulating levels of 18:0, 20:0, and 24:0 were each associated with lower risk of AF. Comparing each of the upper quartiles with the lowest quartile of plasma phospholipid 18:0, the hazard ratios for AF were 0.89 (95% CI: 0.72 to 1.09), 0.77 (95% CI: 0.62 to 0.95), and 0.76 (95% CI: 0.61 to 0.95) (P -trend=0.01) (Table 4). Results for 20:0, 22:0, and 24:0 appeared similar to or stronger than those of 18:0 (Table 5). Restricting analyses to the first 8 years of follow-up did not materially alter results (data not shown). There was no evidence of interaction of age, sex, or hypertension status with each SFA on risk of AF, and associations of SFAs with AF incidence were similar in analyses stratified by sex, age, and hypertension status (data not shown). Results of cross-tabulation analyses suggest no major subgroup effects of age on the associations of each SFA with AF (Tables 6 through 10). Exploratory models that included all 5 SFAs resulted in attenuated hazard ratios that

were no longer significant (data not shown). In exploratory analyses, the associations of 22:0 and 24:0, but not that of 18:0 and 20:0, remained significant with adjustment for 16:0 (data not shown).

Discussion

In this large, prospective, community-based cohort study of older US adults, objective SFA biomarkers were associated with incident AF. Specifically, plasma phospholipid 16:0 was associated with significantly higher risk, whereas 18:0, 20:0, 22:0, and 24:0 were each associated with significantly lower risk. These data support the hypothesis that the health effects of circulating SFAs, or the metabolic determinants of their circulating levels, may differ by chain length.

Individual SFAs have different dietary and metabolic origins. Whereas 16:0 is the most abundant SFA in both diet and circulation, its circulating levels are poorly correlated with dietary intake.^{31,32} This suggests that metabolic pathways are the primary determinant of circulating levels. One key driver of higher circulating 16:0 appears to be de novo lipogenesis, an endogenous enzymatic pathway by which dietary carbohydrates are converted into circulating fatty acids in the presence of low-fat and high-carbohydrate diets.^{6–8,33} Alcohol intake may also drive the endogenous synthesis of 16:0.^{9–11}

Similar to 16:0, SFA 18:0 can be derived from animal products, including beef and hard cheeses,³⁴ but it also can be found in other food sources, such as shea butter, cocoa butter, and chocolate.^{35,36} SFA 18:0 may also be produced endogenously from the elongation of 16:0.³⁷ Recent studies have demonstrated the unique effects of 18:0 on plasma lipids and lipoproteins; unlike other SFAs, including 16:0, that are associated with hypercholesterolemia, 18:0 has no effect on lipid metabolism.^{31,38} Although the biological mechanism underlying these differential effects is unknown, this finding highlights potential, diverse physiological effects and/or underlying metabolic pathways of individual SFAs.

Very long-chain SFAs 20:0, 22:0, and 24:0 are derived from dietary sources, such as peanuts and canola oil, and may also be produced endogenously from the elongation of 18:0 to 20:0, 22:0, and 24:0.^{37,39} Very long-chain SFAs exhibit distinct functions when compared with other long-chain SFAs. Very long-chain SFAs, for example, are known to influence liver homeostasis, retinal function, and anti-inflammatory functions.⁴⁰ Unfortunately, little is known about very long-chain SFA, and more studies are needed to better understand the pathways by which these SFAs may influence human health and disease.

The biological mechanisms by which circulating SFAs may influence risk of AF are largely unknown. Increasing experimental evidence suggests that the long-chain SFA 16:0 may

Table 3. Baseline Characteristics of Study Participants According to Plasma Phospholipid 18:0, 20:0, and 22:0*

Quartile	Quartile of 18:0				Quartile of 20:0				Quartile of 22:0			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
% Total FA, median	12.3	13.1	13.8	14.7	0.41	0.47	0.52	0.59	1.3	1.6	1.8	2.0
Range	8.2 to 12.7	12.7 to 13.4	13.4 to 14.2	14.2 to 18.9	0.26 to 0.44	0.44 to 0.50	0.50 to 0.55	0.55 to 0.82	0.16 to 1.5	1.5 to 1.7	1.7 to 1.9	1.9 to 3.5
N	727	740	714	718	697	710	731	761	706	697	736	760
Age, y	75.5 (5.5)	75.2 (5.3)	74.4 (4.9)	73.8 (4.6)	74.8 (5.1)	74.9 (5.0)	74.8 (5.3)	74.4 (5.1)	75.3 (5.3)	75.4 (5.1)	74.4 (5.2)	73.8 (4.8)
Sex, % male	43.7%	40.5%	36.1%	24.8%	39.2%	40.8%	33.0%	32.9%	36.1%	35.4%	37.6%	36.2%
Race, % white	90.6%	87.7%	86.0%	82.3%	92.1%	89.3%	87.1%	78.8%	91.1%	90.2%	85.9%	80.1%
Education, % >high school	41.5%	36.5%	38.9%	32.7%	36.3%	35.6%	41.6%	36.1%	38.7%	40.0%	35.7%	35.5%
Current smoker, %	10.2	10.3	8.5	9.2	9.5	8.5	10.1	10.1	9.5	10.3	7.7	10.7
Alcohol, drinks/week	0.02 (0 to 2)	0 (0 to 1)	0 (0 to 1)	0 (0 to 1)	0 (0 to 2)	0 (0 to 1.04)	0 (0 to 1)	0 (0 to 0.75)	0.02 (0 to 2)	0 (0 to 1.04)	0 (0 to 1)	0 (0 to 0.41)
Body mass index, kg/m ²	25.4 (4.1)	26.4 (4.9)	27.4 (4.7)	27.9 (4.8)	26.5 (4.2)	26.5 (4.7)	26.6 (4.9)	27.4 (4.9)	26.6 (4.7)	26.5 (4.8)	27.0 (4.9)	27.0 (4.5)
Waist circumference, cm	93.7 (12.6)	95.8 (13.5)	98.9 (13.2)	99.8 (13.3)	97.1 (12.5)	96.7 (13.7)	96.5 (13.7)	97.8 (13.6)	97.2 (13.9)	96.6 (13.8)	97.1 (13.4)	97.2 (12.5)
Diabetes mellitus, %	16.0	16.1	12.2	13.8	20.7	14.4	11.5	12.0	19.7	12.9	10.3	15.3
Treated hypertension, %	33.9	32.7	37.3	42.1	39.9	38.2	35.7	32.5	43.0	36.6	32.3	34.4
Aspirin >2 days in 2 weeks, %	31.7	30.4	30.4	29.3	29.8	30.5	33.3	28.3	29.5	31.7	31.1	29.6
Lipid-lowering drugs, %	4.3	5.1	4.6	8.6	6.3	5.5	5.6	5.3	7.2	6.6	4.9	4.1
Hx of stroke, %	4.1	3.2	4.3	3.6	4.3	3.1	3.7	4.2	4.7	4.0	3.1	3.6
Hx of heart failure, %	1.9	2.2	2.2	2.4	1.6	2.5	2.2	2.4	2.4	2.6	1.6	2.1
Cystatin C, mg/L	1.1 (0.3)	1.1 (0.3)	1.1 (0.3)	1.1 (0.3)	1.1 (0.3)	1.1 (0.3)	1.1 (0.3)	1.1 (0.2)	1.1 (0.3)	1.1 (0.3)	1.1 (0.2)	1.1 (0.3)
eGFR <60 mL/min per 1.73 m ² , %	20.1	19.2	22.1	19.5	24.6	18.4	20.0	18.4	25.3	20.1	18.6	17.4
Carbohydrate, % energy	53.6 (7.4)	53.9 (7.3)	53.9 (7.2)	54.1 (6.9)	54.2 (7.2)	54.0 (7.3)	53.7 (7.1)	53.7 (7.3)	54.5 (7.7)	53.6 (7.1)	54.1 (7.3)	53.3 (6.6)
Total fat, % energy	30.7 (5.6)	30.8 (5.6)	31.0 (5.5)	30.8 (5.5)	30.5 (5.4)	30.8 (5.5)	30.9 (5.4)	31.1 (5.9)	29.9 (5.8)	30.8 (5.3)	30.9 (5.6)	31.7 (5.4)
Saturated fat, % energy	10.1 (2.2)	10.2 (2.2)	10.4 (2.3)	10.2 (2.2)	10.1 (2.1)	10.2 (2.2)	10.2 (2.2)	10.4 (2.4)	10.0 (2.3)	10.2 (2.2)	10.1 (2.2)	10.5 (2.2)
Polysaturated fat, % energy	6.6 (1.9)	6.5 (1.7)	6.6 (1.8)	6.6 (1.7)	6.6 (1.7)	6.5 (1.7)	6.6 (1.8)	6.6 (1.8)	6.3 (1.8)	6.6 (1.7)	6.7 (1.8)	6.7 (1.7)
Peanut intake, servings/week	0.46 (0.14 to 2.5)	0.46 (0.14 to 2.5)	0.46 (0.14 to 2.5)	0.46 (0.14 to 2.5)	0.46 (0.14 to 2.5)	0.46 (0.14 to 2.5)	0.46 (0.14 to 2.5)	0.46 (0.14 to 2.5)	0.46 (0.14 to 2.5)	0.46 (0.14 to 2.5)	0.46 (0.14 to 2.5)	0.46 (0.14 to 2.5)

eGFR indicates estimated glomerular filtration rate; FA, fatty acid; Hx, history.

*Data are mean (SD), percentage or median (interquartile range).

Table 4. Hazard Ratio for Incident Atrial Fibrillation According to Plasma Phospholipid Long-Chain Saturated Fatty Acids

Quartile	Q1	Q2	Q3	Q4	P-trend
16:0					
Person-years	7925.3	7704.3	7181.9	7052.1	
No. of cases	151	172	183	201	
Hazard ratio (95% CI)					
Age and sex adjusted	1.0 (ref)	1.12 (0.90 to 1.39)	1.24 (1.00 to 1.54)	1.47 (1.19 to 1.82)	<0.0001
Multivariate model*	1.0 (ref)	1.12 (0.89 to 1.40)	1.25 (0.99 to 1.56)	1.48 (1.18 to 1.86)	<0.0001
18:0					
Person-years	6821.1	7382.6	7797.3	7862.6	
No. of cases	196	188	160	163	
Hazard ratio (95% CI)					
Age and sex adjusted	1.0 (ref)	0.89 (0.73 to 1.09)	0.76 (0.62 to 0.94)	0.81 (0.65 to 1.00)	0.02
Multivariate model*	1.0 (ref)	0.89 (0.72 to 1.09)	0.77 (0.62 to 0.95)	0.76 (0.61 to 0.95)	0.01

*Adjusted for age, sex, race, clinic, education, smoking, alcohol use, body mass index, waist circumference, physical activity, treated hypertension, diabetes, history of stroke, history of heart failure, and plasma phospholipid long-chain n-3 fatty acids.

increase apoptosis, whereas very long-chain SFAs (20:0, 22:0, and 24:0) may decrease apoptosis.^{41–44} Animal and human studies suggest a role of apoptosis in the pathophysiology of AF, including fibrosis and the progressive remodeling leading to AF,^{45–50} suggesting a possible mechanism by which higher

levels of plasma phospholipid 16:0 may be associated with a higher risk of AF, whereas 20:0, 22:0, and 24:0 may be associated with a lower risk of AF. More research is needed to better understand how circulating SFAs may influence risk of AF.

Table 5. Hazard Ratio for Incident Atrial Fibrillation According to Plasma Phospholipid Very Long-Chain Saturated Fatty Acids

Quartile	Q1	Q2	Q3	Q4	P-trend
20:0					
Person-years	6696.2	7419.0	7791.0	7957.4	
No. of cases	203	175	161	168	
Hazard ratio (95% CI)					
Age and sex adjusted	1.0 (ref)	0.75 (0.61 to 0.92)	0.68 (0.55 to 0.84)	0.71 (0.58 to 0.87)	0.001
Multivariate model*	1.0 (ref)	0.77 (0.63 to 0.95)	0.71 (0.57 to 0.87)	0.78 (0.63 to 0.97)	0.01
22:0					
Person-years	6496.4	6995.9	8034.3	8337.0	
No. of cases	205	172	174	156	
Hazard ratio (95% CI)					
Age and sex adjusted	1.0 (ref)	0.75 (0.61 to 0.92)	0.67 (0.55 to 0.82)	0.61 (0.49 to 0.75)	<0.0001
Multivariate model*	1.0 (ref)	0.75 (0.61 to 0.92)	0.72 (0.58 to 0.89)	0.62 (0.50 to 0.78)	<0.0001
24:0					
Person-years	6445.8	7368.9	7600.1	8448.7	
No. of cases	198	173	175	161	
Hazard ratio (95% CI)					
Age and sex adjusted	1.0 (ref)	0.75 (0.61 to 0.92)	0.71 (0.58 to 0.87)	0.60 (0.49 to 0.74)	<0.0001
Multivariate model*	1.0 (ref)	0.82 (0.67 to 1.01)	0.77 (0.63 to 0.96)	0.68 (0.55 to 0.85)	0.001

*Adjusted for age, sex, race, clinic, education, smoking, alcohol use, body mass index, waist circumference, physical activity, treated hypertension, diabetes, history of stroke, history of heart failure, and plasma phospholipid long-chain n-3 fatty acids.

Table 6. Hazard Ratios for Incident Atrial Fibrillation According to Plasma Phospholipid 16:0 and Age*

	Age <74 y	Age ≥74 y
Plasma phospholipid 16:0 <25.3% total fatty acids	1.00	0.97 (0.72 to 1.30)
Plasma phospholipid 16:0 ≥25.3% total fatty acids	1.27 (1.01 to 1.59)	1.20 (0.90 to 1.61)

Plasma phospholipid 18:0 and age are both stratified at the median.

*Adjusted for age, sex, race, clinic, education, smoking, alcohol use, body mass index, waist circumference, physical activity, treated hypertension, diabetes, history of stroke, history of heart failure, and plasma phospholipid long-chain n-3 fatty acids.

To date, no other published studies have examined the associations of circulating SFAs with incident AF, and results of studies that have examined associations of circulating SFAs with risk factors for AF (eg, hypertension, myocardial infarction, heart failure) have shown inconsistent findings.^{11–17} Two previous studies examined the relationship of circulating 16:0 and 18:0 with cardiovascular events. Results from a population-based retrospective case control study indicated that higher levels of circulating 16:0, but not 18:0, are associated with a higher risk of sudden cardiac arrest. In that study, a 1-SD-higher level of circulating 16:0 was associated with a 38% higher risk.¹⁷ In contrast, a previous analysis in CHS was not able to demonstrate an association of 16:0 and 18:0 with sudden cardiac arrest and showed little evidence of a strong association of these fatty acids with incident coronary heart disease. The study showed, however, that higher levels of circulating 18:1n-7, a fatty acid derived from the desaturation and elongation of 16:0, were associated with a 7-fold higher risk of sudden cardiac arrest.¹¹

Our study has several strengths. CHS is a large, multicenter, longitudinal study in older adults, a population with high risk of AF. The prospective analysis and cohort design

Table 7. Hazard Ratios for Incident Atrial Fibrillation According to Plasma Phospholipid 18:0 and Age*

	Age <74 y	Age ≥74 y
Plasma phospholipid 18:0 <13.4% total fatty acids	1.00	0.89 (0.67 to 1.19)
Plasma phospholipid 18:0 ≥13.4% total fatty acids	0.76 (0.60 to 0.95)	0.78 (0.58 to 1.09)

Plasma phospholipid 18:0 and age are both stratified at the median.

*Adjusted for age, sex, race, clinic, education, smoking, alcohol use, body mass index, waist circumference, physical activity, treated hypertension, diabetes, history of stroke, history of heart failure, and plasma phospholipid long-chain n-3 fatty acids.

Table 8. Hazard Ratios for Incident Atrial Fibrillation According to Plasma Phospholipid 20:0 and Age*

	Age <74 y	Age ≥74 y
Plasma phospholipid 20:0 <0.50% total fatty acids	1.00	0.88 (0.67 to 1.16)
Plasma phospholipid 20:0 ≥0.50% total fatty acids	0.75 (0.60 to 0.94)	0.80 (0.60 to 1.06)

Plasma phospholipid 20:0 and age are both stratified at the median.

*Adjusted for age, sex, race, clinic, education, smoking, alcohol use, body mass index, waist circumference, physical activity, treated hypertension, diabetes, history of stroke, history of heart failure, and plasma phospholipid long-chain n-3 fatty acids.

reduced potential for both recall bias and selection bias. In addition, individual SFAs were measured using an objective biomarker. The use of multiple methods to identify AF (hospital discharge records, ECGs) may lower the possibility of misclassification of AF. Detailed information on demographic and clinical factors was collected using standard instruments, which increased our ability to adjust for confounding. Finally, the community-based enrollment of the cohort increased generalizability.

This analysis also has potential limitations. Plasma phospholipid fatty acids were assessed only at a single time point (1992–1993), and we were unable to account for potential changes in circulating levels of SFAs due to changes in diet or metabolism over the 16-year follow-up. Although the FFQs provided a measure of average daily energy and nutrient intake, many of the foods high in SFAs (eg, canola oil, tropical oils, hard cheese, and nuts) were not included on the FFQs, and we were unable to examine the relationship of circulating SFAs and dietary SFAs. Annual ECGs were performed only through 1999, and it is possible that ascertainment of AF was better during 1992–1999 than during 2000–2008; however, restricting analyses to the first 8 years of follow-up did not

Table 9. Hazard Ratios for Incident Atrial Fibrillation According to Plasma Phospholipid 22:0 and Age*

	Age <74 y	Age ≥74 y
Plasma phospholipid 22:0 <1.67% total fatty acids	1.00	0.90 (0.68 to 1.19)
Plasma phospholipid 22:0 ≥1.67% total fatty acids	0.74 (0.59 to 0.93)	0.74 (0.56 to 1.00)

Plasma phospholipid 22:0 and age are both stratified at the median.

*Adjusted for age, sex, race, clinic, education, smoking, alcohol use, body mass index, waist circumference, physical activity, treated hypertension, diabetes, history of stroke, history of heart failure, and plasma phospholipid long-chain n-3 fatty acids.

Table 10. Hazard Ratios for Incident Atrial Fibrillation According to Plasma Phospholipid 24:0 and Age*

	Age <74 y	Age ≥74 y
Plasma phospholipid 24:0 <1.38% total fatty acids	1.00	0.95 (0.72 to 1.26)
Plasma phospholipid 24:0 ≥1.38% total fatty acids	0.81 (0.65 to 1.02)	0.78 (0.59 to 1.05)

Plasma phospholipid 24:0 and age are both stratified at the median.

*Adjusted for age, sex, race, clinic, education, smoking, alcohol use, body mass index, waist circumference, physical activity, treated hypertension, diabetes, history of stroke, history of heart failure, and plasma phospholipid long-chain n-3 fatty acids.

materially change the results. Because we used ECGs and hospital discharge diagnoses to identify incident AF, newly recognized paroxysmal AF that was managed only in the outpatient setting may have been missed. Conversely, such patients often eventually receive a hospital diagnosis of AF, so some of these AF diagnoses were likely simply delayed rather than missed entirely. Although we adjusted for several factors associated with SFA levels and AF risk, residual confounding by unknown or poorly measured factors is possible. Our analyses assessed the associations of multiple SFAs on risk of AF, increasing the possibility that our findings may be due to chance alone. We decided not to adjust for multiple comparisons in the analyses because the circulating SFAs were moderately to highly correlated. Moreover, the high correlation of some of the circulating SFAs, particularly 22:0 and 24:0, limited our ability to isolate the effects of individual fatty acids on risk of AF, and interpretation of analyses that mutually adjusted for all SFAs is challenging. Finally, our cohort comprised participants aged 65 years or older, and results may not be generalizable to younger populations, in which AF is far less frequent.

In conclusion, the results of this analysis suggest that among older adults, higher levels of circulating 16:0 are associated with a higher risk of AF, whereas higher levels of circulating 18:0, 20:0, 22:0, and 24:0 are associated with lower risk. This study adds to the growing body of evidence suggesting potential differences in the health effects and/or underlying metabolic determinants of different types of circulating SFAs. These novel findings highlight the need for further observational and mechanistic investigation to better understand how circulating SFAs may influence risk of AF.

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