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Associations of Plasma Phospholipid SFAs with Total and Cause-Specific Mortality in Older Adults Differ According to SFA Chain Length^{1–4}

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Abstract

Background: Not much is known about the relations of circulating saturated fatty acids (SFAs), which are influenced by both metabolic and dietary determinants, with total and cause-specific mortality.

Objective: We examined the associations of plasma phospholipid SFAs with total and cause-specific mortality among 3941 older adults from the Cardiovascular Health Study, a population-based prospective study of adults aged ≥ 65 y who were followed from 1992 through 2011.

Methods: The relations of total and cause-specific mortality with plasma phospholipid palmitic acid (16:0), stearic acid (18:0), arachidic acid (20:0), behenic acid (22:0), and lignoceric acid (24:0) were assessed using Cox proportional hazards models.

Results: During 45,450 person-years of follow-up, 3134 deaths occurred. Higher concentrations of the plasma phospholipid SFAs 18:0, 22:0, and 24:0 were associated with a lower risk of total mortality [multivariable-adjusted HRs (95% CIs)] for the top compared with the bottom quintile: 0.85 (0.75, 0.95) for 18:0; 0.85 (0.75, 0.95) for 22:0; and 0.80 (0.71, 0.90) for 24:0. In contrast, plasma 16:0 concentrations in the highest quintile were associated with a higher risk of total mortality compared with concentrations in the lowest quintile [1.25 (1.11, 1.41)]. We also found no association of plasma phospholipid 20:0 with total mortality.

Conclusions: These findings suggest that the associations of plasma phospholipid SFAs with the risk of death differ according to SFA chain length and support future studies to better characterize the determinants of circulating SFAs and to explore the mechanisms underlying these relations. *J Nutr* 2016;146:298–305.

Keywords: saturated fat, plasma phospholipid FAs, SFAs, mortality, elderly

Introduction

SFAs of different chain lengths have unique metabolic and biological processes, and the associations of SFAs with health outcomes may vary by SFA type (1, 2). Circulating SFAs, which can be derived from both endogenous synthesis as well as diet, provide objective biomarkers of specific SFAs of different chain lengths. The 2 major

long-chain SFAs, palmitic acid (16:0) and stearic acid (18:0), are derived from animal products, chocolate, and tropical oils (1, 3, 4) and from hepatic de novo lipogenesis (DNL)¹⁸/FA synthesis,

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⁴ Supplemental Tables 1–9 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at <http://jn.nutrition.org>.

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¹⁸ Abbreviations used: CHD, coronary heart disease; CHS, Cardiovascular Health Study; CVD, cardiovascular disease; DNL, de novo lipogenesis.

especially in the context of low-fat, high-carbohydrate diets (5–11). These FAs are defined as long-chain FAs because they comprise 14–18 carbon atoms. Less is known about metabolic determinants of longer-chain SFAs (i.e., very-long-chain SFAs), such as arachidic acid (20:0), behenic acid (22:0), or lignoceric acid (24:0). These FAs comprise 20–24 carbon atoms and in the diet are primarily derived from canola oil, peanuts, and peanut butter (12, 13).

There is increasing evidence that circulating SFAs may influence the risk of cardiometabolic diseases and mortality. [Although long-chain and very-long chain SFAs are not the only SFAs in circulation (i.e., medium- and odd-chain SFAs are also present but differ from that of endogenously synthesized SFAs), we will refer to 16:0, 18:0, 20:0, 22:0, and 24:0 as simply SFAs henceforth.] Several studies suggest that circulating SFAs 16:0 and 18:0 are positively associated with cardiovascular disease (CVD) or CVD-related events (14–18), diabetes (19, 20), and mortality (21, 22). On the other hand, a handful of recent studies indicate an inverse relation of the longer-chain circulating SFAs (i.e., 20:0, 22:0, and 24:0) with atrial fibrillation (23), diabetes (20), and sudden cardiac arrest (24). To our knowledge, no studies to date have assessed the relations of 20:0, 22:0, and 24:0 with mortality.

We hypothesized that circulating concentrations of 16:0 and 18:0 are positively associated with total and cause-specific mortality and that circulating concentrations of 20:0, 22:0, and 24:0 are negatively associated with total and cause-specific mortality. Using data collected as part of the CHS (Cardiovascular Health Study), a cohort study of risk factors for CVD among adults aged ≥ 65 y from 4 communities in the United States, we investigated the associations of the primary SFAs (16:0 and 18:0) and longer-chain SFAs (20:0, 22:0, and 24:0) with total mortality (primary analyses) and CVD and non-CVD mortality (secondary analyses).

Methods

Study population. The CHS is a prospective multicenter community-based cohort study of CVD and its risk factors among older adults from 4 US counties: Forsyth County, North Carolina; Sacramento County, California; Washington County, Maryland; and Allegheny County, Pennsylvania. Details of the study design and data collection methodology have been described previously (25). In brief, Medicare eligibility lists were used to randomly recruit and enroll ambulatory and noninstitutionalized men and women aged ≥ 65 y. There were 5201 participants enrolled in 1989–1990 and 687 participants (predominantly African American) enrolled in 1992–1993. Participants were followed by annual clinic visits with interim phone calls through 1999 and by phone 2 times per year thereafter. All procedures followed were in accordance with the Helsinki Declaration of 1975 as revised in 1983. Each center's institutional review board approved the study, and all study participants provided written informed consent. The analysis comprised all 3941 study participants with available plasma phospholipid FAs measured using blood samples from 1992–1993 whose characteristics were generally similar to the overall cohort of 5265 participants alive at this study visit (Supplemental Table 1).

Plasma phospholipid SFA measurement. Blood was drawn after a 12-h fast, stored at -70°C until shipped on dry ice to a central laboratory, and then stored at -80°C until analyzed. The SFAs were measured at the Fred Hutchinson Cancer Research Center using stored samples from the 1992–1993 exam. SFAs are extremely stable because of the lack of an oxidation-prone double bond. In addition, most enzyme-catalyzed reactions are temperature-dependent and occur at extremely slow rates at -70°C (26). The Folch methods were used to extract total lipids from plasma (27), and 1-dimensional thin-layer chromatography was then used to separate phospholipids from neutral lipids. To prepare FA methyl esters, phospholipid fractions were directly transesterified using the Lepage and Roy method (28). Individual FA methyl esters were separated using gas chromatography [Agilent Technologies 5890 gas

chromatograph flame ionization detector; Supelco fused SP-2560 silica capillary column (100 m \times 0.25 mm, 0.2 μm); initial 160°C for 16 min, ramp $3^{\circ}\text{C}/\text{min}$ to 240°C , hold 15 min (29)]. Each individual FA is expressed as weight percent of total FAs analyzed. Interassay coefficients of variation were $<3.5\%$ for each of the SFAs of interest.

Mortality assessment. A central CHS events committee adjudicated causes of death using available data from medical records, diagnostic reports, laboratory results, death certificates, and interviews with next of kin. Details of CHS methods for confirmation and classification of death have been reported previously (25, 30). For primary analyses, we were most interested in the relations of the SFAs with total mortality. In secondary analyses, we assessed the relation of each SFA of interest with CVD mortality and non-CVD mortality. In exploratory analyses, we then further subclassified CVD mortality as coronary heart disease (CHD) mortality and non-CVD mortality as deaths from cancer, dementia, infection, or respiratory diseases. All deaths were adjudicated through December 31, 2011.

Assessment of other covariates. The 1992–1993 clinic visit included a comprehensive examination that consisted of a standardized interview, physical examination, laboratory assessment, and diagnostic tests. Standardized questionnaires were administered at the interview to collect information on education, medical history, medication use, smoking, alcohol consumption, physical activity, and self-reported health status. BMI, waist circumference, blood pressure, and biomarkers were assessed using standardized methods as reported previously (25, 31). Alcohol consumption was assessed as drinks per week, with 1 drink equal to a 12-ounce bottle or can of beer, a 6-ounce glass of wine, or a single shot of liquor (~ 14 g of ethanol). Usual dietary intake was estimated using results from FFQs administered in 1989 and 1996 (32, 33). Because the blood samples used to measure plasma phospholipid FAs for this analysis were collected during 1992–1993 we cumulatively updated dietary intake for nutrients included on both FFQs (i.e., total caloric, carbohydrate, and fat intake) among participants who completed both FFQs. Peanut intake was assessed as servings per week, with 1 serving equal to a 28.3-g (1-ounce) serving.

Statistical analyses. This analysis focused on associations of the major long-chain and very-long chain SFAs (from 16:0 to 24:0) that are derived from both diet and endogenous FA synthesis. We did not evaluate 15:0 and 17:0 SFAs, which are biomarkers of dairy fat intake that cannot be synthesized endogenously, because the dietary sources, potential confounders, and potential-effect modifiers of dairy fat and dairy foods are quite different from that of endogenously synthesized SFAs and thus require a separate investigation.

All statistical analyses were performed using Stata version 13.0 (Stata Corp). Plasma phospholipid SFAs were assessed categorically using indicator quintiles. Cox proportional hazards regression was used to examine the HR and 95% CI for the relation of each SFA of interest with total and cause-specific mortality. Participants were censored at the time of death or loss to follow-up (2.5% of study participants lost to follow-up during the 19-y follow-up). A key assumption to Cox models is that hazards are proportional over time. Plotting Schoenfeld residuals is a way to test that the proportional hazards assumption has not been violated. Schoenfeld residuals are specific to each person and covariate and should be independent of time when model assumptions are satisfied. Residual plots for each SFA compared with time were evaluated; no nonrandom patterns were seen, so there was no evidence that the proportional hazards assumption had been violated. A Bonferroni correction was used to adjust for multiple comparisons; the significance threshold used for the current analysis was $P = 0.01$ (based on 5 comparisons for primary analyses). Pearson correlation coefficients were used to measure the degree of correlation between the SFAs of interest.

Covariates were selected a priori based on their potential associations with the SFAs of interest and total mortality using data collected as part of the 1992–1993 examination. These data included age and sex in minimally adjusted models in addition to race (white, African American, other), enrollment site (Bowman Gray, Davis, Hopkins, Pittsburgh), education (no high school, high school/vocational school, college),

smoking (never, past, current), alcohol use (drinks per week), physical activity (kcal/wk), BMI (in kg/m²), waist circumference (cm), treated hypertension (yes/no), prevalent CVD (yes/no), prevalent diabetes (yes/no), self-reported prevalent cancer (yes/no), and self-reported health status (excellent, very good, good, fair, poor) for the primary models.

In exploratory analyses, we assessed the relations of plasma phospholipids 16:0, 18:0, 22:0, and 24:0 with subtypes of CVD and non-CVD mortality, including deaths related to CHD, cancer, dementia, infection, and respiratory diseases. Because previous studies have shown an inverse association of circulating ω -3 FAs with total and cause-specific mortality (34), we performed sensitivity analyses that additionally adjusted each primary model for plasma phospholipid total ω -3 FAs (DHA + docosapentaenoic acid + EPA). Because peanuts contain the SFAs 20:0, 22:0, and 24:0 and previous studies have reported an inverse association of peanut intake with the risk of death (35–39), it is important to evaluate whether the observed associations of circulating SFAs with total mortality are confounded by other components of 1 of their major dietary sources. Thus, we ran sensitivity analyses to further adjust for peanut intake to better understand whether observed associations were independent of peanut intake. Similarly, 16:0 is the primary end product of DNL (16, 40), an endogenous enzymatic pathway by which dietary carbohydrates are converted into circulating FAs in the presence of low-fat and high-carbohydrate diets (10). Because carbohydrate and total fat intake may influence concentrations of 16:0 and affect other pathways for mortality (18), we performed a sensitivity analysis that adjusted for carbohydrate and total fat intake to explore whether the association of 16:0 with total mortality was independent of these dietary factors.

Because circulating SFA concentrations may fluctuate over time, we also performed sensitivity analyses that restricted follow-up to 9.5 y (midpoint) to minimize the misclassification of exposure with increasing follow-up. To address the possibility of reverse causality, we also performed a sensitivity analysis that excluded participants who died during the first 2 years of follow-up. Because age, sex, race, and BMI may influence diet, FA metabolism, and the risk of death, we evaluated in exploratory models the potential interaction of each SFA (modeled continuously) with each of these factors on total mortality. Wald tests were used to evaluate the statistical significance of the multiplicative interaction terms. SFA 16:0 is the main SFA product from DNL, and FAs in the DNL pathway have been shown to be associated with total mortality (21, 22); to better understand whether the associations of longer SFAs with mortality were independent from a biomarker of DNL, we ran exploratory models for the analyses of 18:0, 20:0, 22:0, and 24:0 with total mortality that further adjusted for 16:0. For the analyses of each SFA of interest with CVD mortality, we additionally adjusted the models for other CVD risk factors, including systolic blood pressure, HDL and LDL cholesterol, and lipid-lowering drug use, to see whether these factors influenced the associations of each SFA of interest with CVD mortality.

Missing covariates (<2% for all covariates) were imputed by multiple imputations using data on 16:0, 18:0, 20:0, 22:0, 24:0, age, sex, race, education, smoking, alcohol use, physical activity, dietary carbohydrate intake, total fat intake, polyunsaturated fat intake, saturated fat intake, peanut intake, total caloric intake, BMI, waist circumference, systolic blood pressure, HDL and LDL cholesterol, lipid-lowering drug use, treated hypertension, self-reported health status, CHD, diabetes, and self-reported cancer at the time of the SFA measurement and mortality during follow-up. There were 10 imputation datasets created as part of the analyses. Values are means \pm SDs unless otherwise indicated.

Results

At baseline, the median age was 74 y (range: 65–98 y), 59% were female, and 88% were white. SFA 16:0 was present in the highest proportions (25.4 \pm 1.59 wt% of FAs), followed by SFA 18:0 (13.5 \pm 1.12). Similar to other trace FAs, mean concentrations were lower (wt% <2% of FAs) for 20:0, 22:0, and 24:0. As reported previously (23), there was a wide range of correlations

between each of the SFAs, with the highest correlations between 22:0 and 24:0 ($r = 0.88$) and 20:0 and 22:0 ($r = 0.63$) and the lowest correlations between 16:0 and 20:0 ($r = -0.29$) and 18:0 and 22:0 ($r = 0.15$) ($P < 0.0001$ for all r values) (Supplemental Table 2).

Baseline characteristics of study participants according to the shortest (i.e., SFA 16:0) and longest (i.e., SFA 24:0) SFAs of interest are shown in Table 1. Participants with higher concentrations of 16:0 and 24:0 were more likely to be male and have higher concentrations of education compared with those with lower concentrations of 16:0 and 24:0 (Table 1). The relations of these FAs with other baseline characteristics such as race, lipid-lowering drug use, dietary factors (i.e., consumption of polyunsaturated fat and peanuts), and the prevalence of diabetes, CHD, and hypertension were not in the same direction. For instance, the prevalence of diabetes was positively associated with 16:0 and negatively associated with 24:0. On the other hand, there was no association of circulating concentrations of 16:0 with lipid-lowering drug use, but participants with higher concentrations of 24:0 reported lower lipid-lowering drug use. Peanut consumption was negatively associated with 16:0 and positively associated with 24:0. Supplemental Tables 3 and 4 provide a description of the baseline characteristics of study participants according to concentrations of the other SFAs of interest (i.e., SFAs 18:0, 20:0, and 22:0).

During 45,450 person-years of follow-up, 3134 deaths occurred. Plasma phospholipid 16:0 was positively associated with total mortality in both age/sex and multivariable-adjusted models when the highest quintile of 16:0 was compared to the lowest quintile of 16:0; a 25% higher risk was evident for the top compared with the bottom quintile (Table 2). Associations for the top quintile were generally similar for non-CVD and CVD mortality, although only non-CVD mortality remained statistically significant after adjusting for multiple comparisons. In contrast to the findings for 16:0, higher plasma phospholipids 18:0, 22:0, and 24:0 were each associated with a lower risk of total mortality (Tables 2 and 3). For example, participants in the highest quintile of 18:0 had a 15% lower risk of death compared with those in the lowest quintile [HR (95% CI) = 0.85 (0.75, 0.95); P -trend = 0.001]. The association of 18:0 with mortality was stronger for non-CVD mortality than CVD mortality. SFAs 22:0 and 24:0 were each also associated with lower total mortality, with the strongest association for 24:0 [extreme quintile HR (95% CI) = 0.80 (0.71, 0.90); P -trend <0.0001] (Table 3). For these FAs, the inverse association was attributable to a lower risk of both non-CVD and CVD mortality. We found no statistically significant association of plasma phospholipid 20:0 with total, CVD, or non-CVD mortality (Table 3).

Cancer (30.4%) and dementia (24.6%) were the most common causes of non-CVD death in this population. In exploratory analyses, 16:0 was associated with a higher risk of death from cancer (Supplemental Tables 5–8). SFA 18:0 was associated with a lower risk of death from dementia. We found no statistically significant associations of these SFAs with deaths from infection or respiratory diseases.

In sensitivity analyses that excluded participants who died during the first 2 y of follow-up, censoring the follow-up at 9.5 y or additionally adjusting the primary model for 1) plasma phospholipid n-3 FAs or 2) peanut intake (and total fat and carbohydrate intake for SFA 16:0 and mortality analyses) did not appreciably alter the findings (data not shown). HRs for the associations of each SFA with CVD mortality were not appreciably altered in a model that additionally adjusted for systolic blood pressure, HDL cholesterol, LDL cholesterol, or the use of

TABLE 1 Baseline characteristics of Cardiovascular Health Study participants according to plasma phospholipid 16:0 and 24:0¹

	Quintiles of 16:0					Quintiles of 24:0				
	1	2	3	4	5	1	2	3	4	5
Median weight % total FAs	23.5	24.6	25.3	26.1	27.5	1.04	1.23	1.37	1.51	1.76
Range	19.5, 24.1	24.1, 24.9	24.9, 25.6	25.6, 26.6	26.6, 33.1	0.52, 1.15	1.15, 1.30	1.30, 1.43	1.43, 1.61	1.61, 3.42
<i>n</i>	789	790	786	788	788	791	790	784	788	788
Age, y	74.8 ± 5.3	74.9 ± 5.3	75.2 ± 5.4	75.9 ± 5.4	74.9 ± 5.1	75.8 ± 5.5	75.7 ± 5.5	75.3 ± 5.3	74.7 ± 5.1	74.1 ± 5.0
Sex, % male	30.7	42.8	43.3	46.6	41.5	33.8	38.7	42.2	45.9	44.2
Race, % white	80.1	83.7	89.1	91.4	92.0	90.8	90.0	88.0	88.7	78.7
Education, % >high school	29.5	31.5	40.7	39.5	42.0	33.9	35.6	34.7	40.5	38.6
Current smoker, %	9.0	10.1	8.5	7.5	10.0	7.8	9.7	9.7	8.4	9.5
Alcohol, drinks/wk	0.5 ± 1.7	1.2 ± 3.2	1.4 ± 3.8	2.2 ± 4.6	4.6 ± 7.8	2.1 ± 5.6	1.9 ± 5.1	1.8 ± 4.5	1.9 ± 4.5	2.0 ± 4.5
BMI, kg/m ²	25.9 ± 4.3	26.6 ± 4.7	27.1 ± 4.8	27.1 ± 4.9	27.0 ± 4.5	27.4 ± 5.1	26.8 ± 4.8	26.5 ± 4.6	26.5 ± 4.4	26.5 ± 4.4
Waist circumference, cm	94.6 ± 12.5	96.8 ± 12.8	97.7 ± 13.3	98.6 ± 13.4	98.1 ± 12.9	99.3 ± 14.1	97.3 ± 13.8	96.9 ± 12.4	96.5 ± 12.3	95.9 ± 12.4
Diabetes mellitus, %	9.4	13.1	16.0	17.3	23.3	23.5	13.9	13.5	14.2	14.1
Coronary heart disease, %	21.9	22.3	23.2	23.9	22.8	24.4	25.6	23.0	22.7	18.4
Treated hypertension, %	41.9	39.7	38.6	39.8	44.2	50.2	43.8	38.8	35.8	35.7
Aspirin >2 d in 2 wk, %	38.4	35.0	38.2	37.1	38.9	36.9	37.4	39.3	37.1	36.8
Lipid-lowering medication, %	9.1	6.5	6.0	5.5	8.1	10.6	7.6	6.0	6.4	4.6
Carbohydrates, % energy	54.9 ± 7.1	54.2 ± 6.9	53.9 ± 7.0	54.0 ± 7.1	53.5 ± 7.8	54.4 ± 7.5	54.4 ± 7.3	54.0 ± 7.2	53.8 ± 6.9	53.8 ± 7.1
Total fat, % energy	30.6 ± 5.6	31.0 ± 5.5	30.9 ± 5.4	30.6 ± 5.6	30.1 ± 5.7	30.1 ± 5.6	30.5 ± 5.6	30.8 ± 5.6	30.9 ± 5.4	31.1 ± 5.7
Polyunsaturated fats, % energy	6.7 ± 1.7	6.6 ± 1.8	6.6 ± 1.7	6.5 ± 1.7	6.3 ± 1.8	6.3 ± 1.8	6.5 ± 1.8	6.5 ± 1.7	6.7 ± 1.7	6.7 ± 1.7
Saturated fats, % energy	9.9 ± 2.2	10.2 ± 2.2	10.2 ± 2.2	10.2 ± 2.3	10.1 ± 2.3	10.1 ± 2.3	10.1 ± 2.2	10.2 ± 2.2	10.1 ± 2.1	10.2 ± 2.3
Peanut intake, ² servings/wk	1.6 ± 1.9	1.6 ± 1.8	1.4 ± 1.7	1.3 ± 1.7	1.1 ± 1.5	1.0 ± 1.4	1.2 ± 1.5	1.3 ± 1.5	1.6 ± 1.8	2.0 ± 2.1
Energy intake, kcal/d	2000 ± 576	2000 ± 636	2030 ± 590	1990 ± 581	1980 ± 556	1960 ± 579	1990 ± 549	2000 ± 606	2010 ± 602	2040 ± 601

¹ Values are means ± SDs or percentages, *n* = 3941.² One serving equals 28.3 g (1 ounce).

lipid-lowering drugs (data not shown). We found no evidence of interactions of the SFAs 16:0, 18:0, 20:0, 22:0 or 24:0 with age, sex, race, or BMI on the risk of total mortality, except for an interaction between 16:0 and BMI (*P*-interaction <0.0001). Upon further exploration of this finding, the association of 16:0 and total mortality was limited to participants with a BMI >23.6 (the 25th percentile of BMI among study participants) (Supplemental Table 9). In exploratory analyses, the inverse associations of 22:0 and 24:0, but not 18:0, with total mortality, remained statistically significant after additionally adjusting for 16:0. For example, the extreme-quintile HRs for 22:0 and 24:0 were 0.89 (95% CI: 0.78, 0.99; *P*-trend = 0.004) and 0.83 (95% CI: 0.74, 0.94; *P*-trend <0.0001), respectively.

Discussion

In this large prospective study of older US adults, plasma phospholipids 18:0, 22:0, and 24:0 were each inversely associated with total mortality. On the other hand, 16:0 was positively associated with total mortality among participants with the highest circulating concentrations only. These findings suggest that the magnitude and direction of the relation of individual SFAs and risk of death differ according to the chain length of the individual SFAs.

SFAs are components of ceramides, which play a role in apoptosis (i.e., cell death) (41), oxidative stress and endothelial dysfunction (42), inflammation (42), lipotoxicity, and insulin resistance (43, 44). However, whereas ceramides with SFA 16:0 promote apoptosis, experimental studies suggest that ceramides with 20:0, 22:0, and 24:0 may prevent apoptosis (2, 45–47). Because apoptosis may promote the development of many chronic diseases, such as cancer, neurodegenerative disorders,

and heart disease (48), this may partly explain the positive association of 16:0 with total mortality and the negative associations of 22:0 and 24:0 with total mortality if circulating SFAs reflect the SFA composition of endogenous ceramides. Interestingly, when we subclassified types of death (i.e., non-CVD deaths, CVD deaths) in secondary analyses, the associations of 16:0 and 18:0 and total mortality were stronger for non-CVD than CVD deaths, whereas the associations of 22:0 and 24:0 were driven by both non-CVD and CVD deaths. This finding is difficult to explain and supports the need for further investigation of the associations of individual SFAs on disease risk.

In exploratory analyses, we found suggestion that BMI may influence the relation of 16:0 and mortality; the association of 16:0 with mortality were limited to participants with a BMI >23.6 (above the 25th percentile for BMI). This was an incidental finding. Because 16:0 can be synthesized by DNL (5–11), this finding is plausible if participants with a higher BMI (and at a higher risk of death) consume low-fat diets.

Previous analyses in the CHS indicate divergent associations of circulating concentrations of individual *n*–3 FAs, *n*–6 FAs, and *trans* FAs with mortality (34, 49, 50). To our knowledge, few studies to date have examined the relations of circulating concentrations of SFAs with total or cause-specific mortality. Our findings are consistent with 2 previous studies in Sweden and England that indicated positive associations of circulating 16:0 with total mortality (21, 22). On the other hand, the Swedish study also reported 1) a positive association of circulating 16:0 with CVD mortality and 2) no association of 18:0 with total mortality (21). Underlying differences between the populations studied may at least partly explain inconsistencies in study results. For instance, compared with CHS study participants, participants in the Swedish study were young males (all participants were aged 50 y at baseline) and were more likely to smoke

TABLE 2 Total and cause-specific mortality according to plasma phospholipid 16:0 and 18:0 among Cardiovascular Health Study participants¹

	Quintiles of concentrations					P-trend
	1	2	3	4	5	
16:0						
Total mortality						
Person-years	9589	9407	9086	8733	8635	
Cases, <i>n</i>	601	617	617	643	656	
Age- and sex-adjusted	1.0 (ref)	1.02 (0.91, 1.14)	1.03 (0.92, 1.15)	1.06 (0.95, 1.18)	1.26 (1.12, 1.41)	<0.0001
Additionally adjusted ²	1.0 (ref)	1.03 (0.91, 1.15)	1.07 (0.95, 1.20)	1.01 (0.90, 1.14)	1.25 (1.11, 1.41)	<0.0001
Non-CVD mortality						
Cases, <i>n</i>	351	396	377	396	394	
Age- and sex-adjusted	1.0 (ref)	1.13 (0.98, 1.30)	1.09 (0.94, 1.26)	1.14 (0.98, 1.31)	1.31 (1.14, 1.52)	0.001
Additionally adjusted ²	1.0 (ref)	1.15 (0.99, 1.33)	1.13 (0.98, 1.32)	1.10 (0.94, 1.28)	1.30 (1.11, 1.51)	0.001
CVD mortality						
Cases, <i>n</i>	250	220	239	245	262	
Age- and sex-adjusted	1.0 (ref)	0.86 (0.71, 1.03)	0.94 (0.78, 1.12)	0.94 (0.79, 1.13)	1.18 (0.99, 1.40)	0.04
Additionally adjusted ²	1.0 (ref)	0.85 (0.70, 1.02)	0.97 (0.81, 1.17)	0.88 (0.73, 1.06)	1.18 (0.98, 1.43)	0.10
18:0						
Total mortality						
Person-years	8274	8744	9092	9573	9767	
Cases, <i>n</i>	665	645	631	597	596	
Age- and sex-adjusted	1.0 (ref)	0.92 (0.83, 1.03)	0.92 (0.82, 1.03)	0.84 (0.75, 0.94)	0.90 (0.80, 1.01)	0.016
Additionally adjusted ²	1.0 (ref)	0.93 (0.83, 1.04)	0.92 (0.82, 1.03)	0.83 (0.74, 0.93)	0.85 (0.75, 0.95)	0.001
Non-CVD mortality						
Cases, <i>n</i>	410	409	389	357	349	
Age- and sex-adjusted	1.0 (ref)	0.94 (0.82, 1.08)	0.91 (0.79, 1.04)	0.79 (0.69, 0.92)	0.82 (0.71, 0.95)	0.001
Additionally adjusted ²	1.0 (ref)	0.92 (0.80, 1.06)	0.91 (0.79, 1.05)	0.79 (0.68, 0.92)	0.78 (0.67, 0.91)	<0.0001
CVD mortality						
Cases, <i>n</i>	254	236	241	239	246	
Age- and sex-adjusted	1.0 (ref)	0.89 (0.75, 1.07)	0.94 (0.79, 1.12)	0.91 (0.77, 1.09)	1.03 (0.86, 1.23)	0.73
Additionally adjusted ²	1.0 (ref)	0.95 (0.79, 1.14)	0.93 (0.78, 1.12)	0.90 (0.74, 1.08)	0.96 (0.79, 1.16)	0.52

¹ Values are HRs (95% CIs), *n* = 3941. Data were analyzed using Cox regression. CVD, cardiovascular disease; ref, reference.

² Additionally adjusted for race, clinic, education, smoking, alcohol use, BMI, waist circumference, physical activity, treated hypertension, prevalent diabetes, prevalent cardiovascular disease, self-reported prevalent cancer, and self-reported health status at baseline.

at baseline (51% reported smoking). To our knowledge, no published studies have examined the association of circulating 20:0, 22:0, or 24:0 with total or cause-specific mortality. However, our findings are consistent with a study that reported an inverse relation of circulating concentrations of the SFAs 20:0, 22:0, and 24:0 with sudden cardiac arrest (24); although our analyses did not assess sudden cardiac arrest as an outcome of interest, it is associated with a high risk of mortality. In addition, 2 recent studies show divergent associations of 16:0 compared with 20:0, 22:0, and 24:0 on the risk of atrial fibrillation (51) and diabetes (20).

Dietary sources of 16:0 and 18:0 include meats, hard cheeses, chocolate, and tropical oils, whereas 20:0, 22:0, and 24:0 are primarily found in peanuts and canola oil. Dietary intake of foods high in 16:0 and 18:0 have shown little influence on endogenous circulating or tissue concentrations of these SFAs. This may be because a meaningful proportion of dietary SFAs are oxidized for energy after consumption. In addition, 16:0 and 18:0 are end products of DNL, which is driven by high doses of rapidly digested carbohydrates as well as fructose. For this reason, low correlations of food sources of 16:0 and 18:0 with circulating 16:0 and 18:0 are commonly seen. For instance, in the EPIC (European Prospective Investigation into Cancer and Nutrition) study, the intake of sugar-sweetened beverages and potatoes (both drivers of DNL) were more highly correlated

with circulating 16:0 and 18:0 concentrations than dietary sources of these SFAs (20). In a recent randomized trial, progressively higher intakes of dietary SFAs across a very large range had little effect on circulating 16:0 and 18:0 concentrations, whereas progressively higher intakes of carbohydrates activated DNL, as evidenced by progressively higher 16:1n-7 (a biomarker of DNL) (52). However, it is largely unknown whether circulating SFAs 20:0, 22:0, and 24:0 are more heavily influenced by diet or endogenous production, and more research is needed to better understand the relations of circulating 20:0, 22:0, and 24:0 with diet.

This study has several strengths. CHS is a large community-based cohort study of CVD and its risk factors in elderly adults, a population with a high risk of mortality. The detailed and standardized methods for data minimize potential recall bias. A biomarker was used to measure plasma phospholipid SFAs; this provides a direct estimate of endogenous exposure to SFAs. The available detailed data on demographic, behavioral, and health factors maximized our capacity to control for potential confounders.

This investigation also has limitations. Plasma phospholipid SFAs were only measured at the 1992–1993 exam, and we were unable to adjust for changes in SFA concentrations over time. We adjusted for demographic, behavioral, and clinical factors that may be related to plasma phospholipid SFAs and total or

TABLE 3 Total and cause-specific mortality according to plasma phospholipid 20:0, 22:0, and 24:0 among Cardiovascular Health Study Participants¹

	Quintiles of concentrations					P-trend
	1	2	3	4	5	
20:0						
Total mortality						
Person-years	8458	9141	9180	9255	9415	
Cases, <i>n</i>	661	627	629	611	606	
Age- and sex-adjusted	1.0 (ref)	0.84 (0.75, 0.94)	0.86 (0.77, 0.96)	0.84 (0.75, 0.93)	0.86 (0.77, 0.96)	0.02
Additionally adjusted ²	1.0 (ref)	0.92 (0.83, 1.03)	0.92 (0.82, 1.03)	0.92 (0.82, 1.03)	0.94 (0.83, 1.05)	0.30
Non-CVD mortality						
Cases, <i>n</i>	383	387	388	376	380	
Age- and sex-adjusted	1.0 (ref)	0.89 (0.77, 1.03)	0.91 (0.79, 1.05)	0.88 (0.76, 1.01)	0.92 (0.80, 1.06)	0.28
Additionally adjusted ²	1.0 (ref)	0.96 (0.83, 1.10)	0.96 (0.83, 1.11)	0.95 (0.82, 1.10)	0.98 (0.84, 1.13)	0.78
CVD mortality						
Cases, <i>n</i>	278	238	240	235	225	
Age- and sex-adjusted	1.0 (ref)	0.77 (0.64, 0.91)	0.79 (0.67, 0.94)	0.78 (0.65, 0.93)	0.78 (0.66, 0.94)	0.02
Additionally adjusted ²	1.0 (ref)	0.87 (0.73, 1.04)	0.86 (0.72, 1.03)	0.88 (0.73, 1.05)	0.87 (0.73, 1.05)	0.18
22:0						
Total mortality						
Person-years	8096	8432	9360	9738	9823	
Cases, <i>n</i>	674	651	621	597	591	
Age- and sex-adjusted	1.0 (ref)	0.89 (0.80, 0.99)	0.77 (0.69, 0.86)	0.71 (0.63, 0.79)	0.78 (0.70, 0.87)	<0.0001
Additionally adjusted ²	1.0 (ref)	0.90 (0.80, 1.00)	0.86 (0.77, 0.97)	0.75 (0.67, 0.84)	0.85 (0.75, 0.95)	<0.0001
Non-CVD mortality						
Cases, <i>n</i>	401	393	388	354	378	
Age- and sex-adjusted	1.0 (ref)	0.90 (0.78, 1.03)	0.80 (0.69, 0.92)	0.70 (0.60, 0.80)	0.83 (0.72, 0.95)	<0.0001
Additionally adjusted ²	1.0 (ref)	0.90 (0.78, 1.03)	0.87 (0.76, 1.01)	0.74 (0.64, 0.86)	0.88 (0.76, 1.01)	0.006
CVD mortality						
Cases, <i>n</i>	272	258	232	242	212	
Age- and sex-adjusted	1.0 (ref)	0.88 (0.74, 1.04)	0.73 (0.61, 0.86)	0.73 (0.61, 0.86)	0.72 (0.60, 0.86)	<0.0001
Additionally adjusted ²	1.0 (ref)	0.90 (0.76, 1.07)	0.85 (0.71, 1.02)	0.77 (0.65, 0.92)	0.80 (0.66, 0.96)	0.003
24:0						
Total mortality						
Person-years	8126	8713	9003	9674	9934	
Cases, <i>n</i>	672	656	615	599	592	
Age- and sex-adjusted	1.0 (ref)	0.86 (0.77, 0.96)	0.78 (0.70, 0.87)	0.68 (0.61, 0.76)	0.70 (0.63, 0.79)	<0.0001
Additionally adjusted ²	1.0 (ref)	0.93 (0.83, 1.04)	0.83 (0.75, 0.93)	0.79 (0.71, 0.89)	0.80 (0.71, 0.90)	<0.0001
Non-CVD mortality						
Cases, <i>n</i>	401	387	376	364	386	
Age- and sex-adjusted	1.0 (ref)	0.85 (0.74, 0.98)	0.80 (0.69, 0.92)	0.69 (0.60, 0.80)	0.76 (0.66, 0.88)	<0.0001
Additionally adjusted ²	1.0 (ref)	0.90 (0.78, 1.04)	0.83 (0.72, 0.96)	0.79 (0.68, 0.91)	0.85 (0.73, 0.98)	0.006
CVD mortality						
Cases, <i>n</i>	270	268	239	234	205	
Age- and sex-adjusted	1.0 (ref)	0.88 (0.74, 1.04)	0.76 (0.64, 0.90)	0.67 (0.56, 0.80)	0.61 (0.51, 0.74)	<0.0001
Additionally adjusted ²	1.0 (ref)	0.98 (0.83, 1.16)	0.85 (0.71, 1.01)	0.81 (0.67, 0.97)	0.72 (0.60, 0.87)	<0.0001

¹ Values are HRs (95% CIs), *n* = 3941. Data were analyzed using Cox regression. CVD, cardiovascular disease; ref, reference.

² Additionally adjusted for race, clinic, education, smoking, alcohol use, BMI, waist circumference, physical activity, treated hypertension, prevalent diabetes, prevalent cardiovascular disease, self-reported prevalent cancer, and self-reported health status at baseline.

cause-specific mortality in the primary analysis, but there may be residual confounding by unmeasured or imprecisely measured factors. It is also possible that some participants may have changed their lifestyle over time. However, in the CHS, the correlations of self-reported smoking or alcohol use at baseline (1992–1993) with self-reported smoking or alcohol use at the 1998–1999 exam are $r = 0.91$ and 0.71 , respectively. This is consistent with what we know about the general population—lifestyle habits in most people remain relatively constant over time. In addition, the strong correlation of some of the plasma phospholipid SFAs (e.g., 22:0 and 24:0) makes it challenging to

interpret the independent associations of each individual SFA with total and cause-specific mortality. Finally, because CHS participants were aged ≥ 65 y at baseline, it is unclear whether these findings are generalizable to younger populations. However, the broad recruitment strategy maximized generalizability to older adults, the population at highest risk for mortality and CVD, making the findings particularly timely and relevant given the growing number of elderly and older adults in the United States.

In conclusion, our findings suggest differential associations between plasma phospholipid SFA concentrations and the risk of total mortality among older adults; higher concentrations of

plasma phospholipid 16:0 were associated with a higher risk of death, whereas higher 18:0, 22:0, and 24:0 concentrations were associated with a lower risk of death. These findings support future efforts to better characterize determinants of circulating SFAs and to explore pathways that explain how circulating SFAs may influence the risk of death.

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AMF, DM, DSS, and RNL designed the research; DM, DSS, and BMP provided essential materials necessary for research; AMF analyzed the data; BM, CS, and DS were the statisticians on the project and supervised the statistical methods; AMF and RNL wrote the paper; DM, DSS, IBK, BM, BMP, EBR, CS, FMS, XS, NS, and DS participated in all analyses and revised drafts of the paper; and AMF had primary responsibility for the final content. All authors read and approved the final manuscript.

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