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## Assessment of Plasma Phospholipid Very-long-chain Saturated Fatty Acid Levels and Healthy Aging: The Cardiovascular Health Study

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## Abstract

**IMPORTANCE**—Identifying novel factors that protect against age-related diseases and promote healthy aging is critical to public health. Higher levels of circulating very-long-chain saturated fatty acids (VLSFAs) are integrated biomarkers of diet and metabolism shown to have beneficial associations in cardiovascular disease and total mortality, but whether they are associated with overall healthy aging is unknown.

**OBJECTIVE**—The objective of the study was to examine the association of circulating levels of three VLSFAs with unhealthy aging events, including incident chronic disease (cardiovascular disease, cancer, lung disease or severe kidney disease), physical dysfunction and cognitive decline.

**DESIGN**—The study design was a prospective cohort study utilizing 1992–2014 data from the Cardiovascular Health Study (CHS).

**SETTING**—The CHS is a multicenter, population-based study of cardiovascular disease among older adults.

**PARTICIPANTS**—Among the 4559 CHS participants with available fatty acid data, we excluded 1879 participants who had an age-related event before their first measurement. The remaining 2680 participants were included in the analyses.

**MAIN OUTCOMES AND MEASURES**—Plasma phospholipid VLSFA levels were measured by thin layer chromatography followed by gas chromatography. The main outcome was the hazard ratio of an incident unhealthy aging event associated with serial measures of plasma arachidic acid (20:0), behenic acid (22:0), and lignoceric acid (24:0).

**RESULTS**—Among the 2680 study participants, the mean age was 74.7 years old at entry and 36.4% were male. During a median 7.5 years of follow up, 2484 participants experienced an unhealthy event. Compared with the lowest quintile, levels of 22:0 in the highest quintile of the fatty acid distribution were associated with lower risk of an unhealthy event (HR, 0.86; 95% CI, 0.74–0.97; *P*trend, 0.01), after adjustment for demographics, lifestyle factors, and clinical conditions. In analogous comparisons, levels of 24:0 were similarly associated with lower risk (HR, 0.84; 95% CI, 0.73–0.95; *P*trend, 0.001).

**CONCLUSIONS AND RELEVANCE**—Higher levels of circulating 22:0 and 24:0 are associated with lower risk of unhealthy aging events. These results highlight the need to explore determinants of circulating VLSFA for potential novel efforts to promote healthy aging.

## Introduction

With increasing life expectancy, the population of older adults is growing rapidly worldwide.<sup>1</sup> However, increased longevity does not necessarily translate into an increase in healthy life span, as older adults experience high rates of cardiovascular and other chronic

diseases.<sup>2</sup> Identifying novel factors that protect against age-related disease and promote healthy aging is critical to public health.

Higher levels of circulating very long-chain saturated fatty acids (VLSFAs), saturated fatty acids with 20 carbons or more, are integrated biomarkers of diet and metabolism that are associated with lower risk of heart failure,<sup>3,4</sup> atrial fibrillation,<sup>5</sup> and diabetes,<sup>6–8</sup> which are chronic diseases that contribute to unhealthy aging, and lower risk of sudden cardiac arrest<sup>9</sup> and total mortality.<sup>10</sup> These widely beneficial associations led us to hypothesize that higher circulating levels of VLSFAs may be broadly associated with a greater likelihood of healthy aging.

We used data from the Cardiovascular Health Study (CHS), a prospective cohort study of risk factors for cardiovascular disease among older adults,<sup>11</sup> to examine the associations of plasma phospholipid levels of three circulating VLSFAs, arachidic acid (20:0), behenic acid (22:0), and lignoceric acid (24:0), measured at the current study baseline and up to 2 more times during follow-up, with risk of an incident unhealthy aging event.

## Methods

### Study Population

CHS is a prospective, population-based cohort study of cardiovascular disease among older adults.<sup>11</sup> Participants were recruited from four U.S. communities (Forsyth County, North Carolina; Sacramento County, California; Washington County, Maryland; Allegheny County, Pennsylvania) from a random sample generated from the Health Care Financing Administration files. Among eligible adults who were contacted, 57% agreed to participate. The cohort consists of 5201 non-institutionalized men and women, aged 65 y, recruited in 1989 through 1990, plus an additional 687 predominantly Black participants recruited in 1992 through 1993. The study was conducted using data from 1992 to 2014 and participants were followed for a median follow up of 7.5 years. Race was classified by self-identification. Each center's institutional review board approved the study, and all participants provided informed written consent.

Plasma phospholipid fatty acids were measured up to 3 times: on blood drawn in 1992 through 1993, the study baseline (N = 3941), on blood drawn in 1998 through 1999 (N = 2609), and on blood drawn in 2005 through 2006 (N = 933). Among the 4559 participants with available fatty acid data from at least one time point, we excluded 1879 participants who had an age-related event before their first fatty acid measurement. The remaining 2680 participants were included in the analyses. Among these, 2434 had fatty acid data in 1992–1993, 1696 in 1998–1999, and 700 in 2005–2006.

### Plasma phospholipid fatty acids

Blood was drawn after 8-h fasting, and plasma specimens were stored at  $-70^{\circ}\text{C}$ . Plasma lipids were extracted by the method of Folch.<sup>12</sup> Phospholipids were separated from other lipids by thin-layer-chromatography. Fatty acid methyl esters were prepared by direct transesterification of the phospholipid fraction<sup>13</sup> and separated by gas chromatography using a fused-silica 100-m capillary column as previously described.<sup>14</sup> Fatty acids were expressed as

a weight percentage of total fatty acids. Inter-assay coefficients of variation for the VLSFA measurements were 3.5%.

### Ascertainment of unhealthy aging events

Participants were followed by means of annual study-clinic examinations with interim phone contacts for 10 years and telephone contacts every 6 months thereafter. Cardiovascular events were adjudicated by a centralized Event Committee. Unhealthy aging was defined based on the established definition in CHS, as previously described,<sup>15</sup> and shown in Table S1. Briefly, we defined an unhealthy event as any of the following: incident cardiovascular disease (myocardial infarction, heart failure, stroke, transient ischemic attack, or claudication), incident severe kidney disease (estimated glomerular filtration rate <10 or dialysis), incident chronic obstructive pulmonary disease, incident cancer, a decrease in cognition (first Mini Mental State Examination score  $\geq$  80), or an increase in difficulties of activities of daily living (first record of difficulty with  $\geq$  1 daily living activity).

### Risk Factors

Information on medical history, medications, life style, and clinical risk factors was collected at annual clinic visits as previously reported.<sup>11</sup> Diabetes was defined by glucose  $\geq$  126 mg/dL when participants reported fasting  $\geq$  8 h before venipuncture, glucose  $\geq$  200 mg/dL when fasting was <8 h, or use of insulin or oral hypoglycemic medication. Body mass index was calculated as body weight (kg) divided by height squared (m<sup>2</sup>). Waist circumference was measured at the umbilicus. Lipids, glucose, insulin, and inflammatory biomarkers were assessed on fasting blood samples using enzymatic methods.<sup>11</sup> The self-reported depression score of 1 to 10 was based on the 10-item Centers for Epidemiological Studies' Depression Scale.<sup>16</sup>

### Statistical Analysis

Baseline (unadjusted) demographic characteristics, cardiometabolic risk factors, and lifestyle habits for the study population were summarized according to quintiles of 24:0. We used a Cox proportional-hazards model to evaluate the association between time-varying VLSFA levels, adjusting for time-varying covariates (updated at each fatty acid measurement) and the likelihood of unhealthy aging. Time-to-event was calculated as the time elapsed between VLSFA measurement and the earliest of: ascertainment of first unhealthy event, death, or date of last follow-up in 2014. The functional forms of the associations between VLSFAs and unhealthy aging were evaluated using natural cubic splines, and tests for non-linearity were performed by comparing the spline model to a linear model using the likelihood ratio test. Quintiles of VLSFA levels were defined from the baseline distributions. Comparisons between quintiles of VLSFA levels were evaluated using time-dependent indicator variables, with tests for trend based on a time-dependent linear variable for quintile. The associations were adjusted for age, race (Black, non-Black), sex, field center, education (less than high school, high school graduates, some college, college graduates), physical activity (number of city blocks walked in the previous week), body mass index, waist circumference, alcohol consumption, smoking status (never, former, current), self-reported health (excellent/very good, good, fair/poor), prevalent diabetes, systolic blood pressure, use of hypertensive medications, and depression score. Modification of the association of VLSFA with the

risk of an unhealthy event was evaluated for age (linear), sex, body mass index (BMI, linear), and prevalent diabetes with statistical significance assessed via the Wald test for the interaction term, in models where VLSFAs were modeled linearly.

Several sensitivity analyses were performed. To assess the role of potential mediators, we fit a model additionally including variables for triglycerides and LDL and one further adjusted for 16:0. To assess the impact of other factors that could influence the associations, we fit separate models adjusting for i.) plasma phospholipid eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA); ii) lipid-lowering medications; and iii.) the sum of plasma phospholipid long chain saturated fatty acids (14:0+16:0+18:0). Finally, to examine whether reverse causation might have created these associations, we performed analyses excluding the first two years of events.

Missing covariates (<4% for all covariates) were imputed using data on age, gender, race, education, smoking, alcohol use, BMI, physical activity, self-reported health status, and prevalent coronary heart disease at baseline, as previously described.<sup>15</sup> Results with imputed data are presented. The results were unchanged from analyses that excluded participants with missing values.

Analyses were performed using Stata version 14.0 (StataCorp, College Station, Texas) R version 4.05 (Vienna, Austria).

This report follows the STROBE guidelines for reporting methods, findings and study limitations for cohort studies.

## Results

Participant mean age (SD) was 74.7 (4.8) years old at entry and 36.4% were male. Median levels of the plasma phospholipids 24:0, 22:0, and 20:0 were 1.38%, 1.66%, and 0.50% of total fatty acids, respectively. Baseline characteristics of the study participants across quintiles of 24:0 are shown in Table 1. Participants in the highest quintile of 24:0 were more likely to be male, Black, college graduates, and current smokers, with higher levels of LDL and physical activity, but they were less likely to have diabetes and showed lower average levels of triglycerides.

During up to 23 years of follow-up, 2484 participants in the previously healthy population experienced an event of unhealthy aging. The distribution of the first events is shown in Table 2, with the most frequent first event being CVD and the least frequent being severe kidney disease. Higher levels of plasma phospholipid 24:0 and 22:0 were each associated with lower risk of unhealthy aging after adjustment for demographics, adiposity measures, physical activity, smoking, alcohol, diabetes, self-reported health, hypertension, and depression score (Table 3). A one standard deviation higher value of 24:0 was associated with 15% lower risk of unhealthy aging (HR: 0.85, 95% CI: 0.77–0.94). Analyses using cubic splines show the associations with lower risk were linear over most of the data, as suggested from the quintile analyses shown in Table 3, with possibly higher risk at very high levels of 22:0 and 24:0 (Figure 1 and Figure S1). However, tests of non-linearity comparing cubic splines with linear models did not show strong evidence of non-linearity

(Table S2). Further adjustments for levels of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA), which may be associated with healthier aging,<sup>17</sup> for the potential mediators triglycerides and LDL, for lipid lowering medications and for the sum of plasma phospholipid long chain saturated fatty acids (14:0+16:0+18:0) did not appreciably change the results (Tables S3 – S6). Adjustment for the combination of triglycerides, LDL and plasma phospholipid 16:0 attenuated the associations slightly (Table 4). Finally, excluding the unhealthy aging events that occurred in the first two years to assess the possibility of reverse causation, did not change the study results (Table S7).

The associations of each plasma phospholipid VLSFA with unhealthy aging did not vary according to age, sex, and BMI (smallest observed *P* for interaction: 0.22). However, we found significant interactions between 20:0 and 22:0 and prevalent diabetes (*p* values for interaction: 0.002 and 0.01, respectively). We observed associations of 20:0 and 22:0 with lower risk of unhealthy aging in the larger group of participants without diabetes, but not in the smaller group of participants with diabetes (Table S8).

## Discussion

For the first time, we have demonstrated in a large prospective cohort that higher levels of plasma phospholipid VLSFAs may be associated with improved healthy life span as shown by lower risk of unhealthy aging events. The strongest association was with 24:0, lignoceric acid. When compared with the lowest quintile, the quintile with the highest levels of 24:0 was associated with a 16% lower risk of an unhealthy aging event (95% CI, 5%–27%), after adjustment for demographics, lifestyle factors, and diabetes.

The primary driver of incident unhealthy aging events in this cohort was cardiovascular disease, followed by declining physical function, cancer, and declining cognition. Higher VLSFAs have beneficial associations with mortality risk,<sup>10</sup> multiple metabolic and inflammatory parameters,<sup>18–21</sup> and a wide range of incident cardiovascular and metabolic diseases such as heart failure,<sup>4</sup> atrial fibrillation,<sup>5</sup> and diabetes.<sup>7,8</sup> Further studies will be needed to investigate potential relationships of VLSFAs to cognition and other age-related conditions. Our purpose here was to study healthy aging, considered as living without chronic disease and with intact physical and mental functions.

Identifying people resistant to the effects of aging compared to peers, and identifying potentially modifiable determinants of this resistance are both of great public health importance.

The univariate association of higher levels of VLSFAs with male sex, Black race, smoking, and an increase in LDL is surprising, as none of these factors is associated with healthy aging. Considering that LDL has a causal effect on CAD,<sup>22</sup> it is interesting that higher levels of VLSFAs have consistent and beneficial associations with risk of heart disease, including CAD,<sup>23,24</sup> despite their LDL association. There are also strong negative associations between VLSFA levels and levels of triglycerides and 16:0 palmitate, markers of de novo lipogenesis,<sup>20</sup> and we previously reported that these markers mediate the associations of VLSFAs with lower risk of diabetes.<sup>7,8</sup> However, adjusting for levels of triglycerides, 16:0

and LDL or prevalent diabetes did not negate the associations between VLSFAs and healthy aging. Interestingly, we found significant effect modification by diabetes, and no benefits of VLSFAs on healthy aging in patients with prevalent diabetes. While this interaction should be interpreted cautiously until replicated, it further illustrates the complex physiological associations of these compounds.

VLSFAs may influence the aging process through multiple mechanisms. They are major components of ceramides and sphingomyelins, lipids that contain one fatty acid acylated to a sphingoid backbone.<sup>25</sup> Ceramides, specifically, are known for their role in apoptosis<sup>26</sup> and inflammation<sup>27</sup> and appear to contribute to the pathogenesis of a broad range of disorders including insulin resistance and atherosclerosis.<sup>28–30</sup> Interestingly, in experimental cell and animal studies, ceramides that contain a VLSFA may actually counteract this tendency by having biological activities opposite from ceramides that contain the shorter saturated fatty acid 16:0, palmitate.<sup>31,32</sup> We speculate that VLSFAs may have a positive effect on healthy aging by lowering endogenous levels of shorter-chain ceramides.

The sources of circulating VLSFAs are both dietary and metabolic. Dietary sources include peanuts, macadamia nuts, and canola oil.<sup>33</sup> Small short-term feeding trials have shown an increase in circulating levels of VLSFAs with consumption of both macadamia nuts<sup>34</sup> and peanut butter,<sup>35</sup> illustrating that dietary intake may directly influence circulating levels of VLSFAs. We reported previously that higher levels of 22:0 and 24:0 were associated with greater peanut intake measured 3 years earlier.<sup>8</sup> VLSFA may also be synthesized endogenously from 18:0 by elongases, such as the ubiquitously distributed ELOVL1.<sup>36</sup> In agreement with the predominant occurrence of VLSFAs in ceramides and other sphingolipids, ELOVL1 appears to be co-regulated with ceramide synthase 2, which produces ceramide by addition of a VLSFA to sphingosine.<sup>37</sup> Further, we have previously shown an association of circulating VLSFAs with genetic variation in the sphingolipid synthesis pathway.<sup>38</sup> The extent to which dietary intake and endogenous metabolism contribute to circulating levels of VLSFA is currently unknown.

### **Study Limitations and strengths.**

The study has several limitations and strengths. This is an observational study and causality cannot be established, as residual confounding by unknown factors is possible. However, the results were robust to adjustment for multiple characteristics. Reverse causality is also of potential concern in association studies<sup>39</sup> and would operate for example if frailty led to low levels of VLSFAs. However, we eliminated participants with a wide range of medical conditions and symptoms in this study of incident unhealthy aging, and examined the association of VLSFAs in a particularly healthy group at entry. We also observed stronger associations in the healthier subgroup without prevalent diabetes. Furthermore, we did not see a weakening of the associations over time, and exclusion of the first two years of follow-up did not remove or attenuate the observed associations, suggesting reverse causation does not account in large part for the observed associations. The study was conducted among older adults and the results may not be generalizable to other populations; however, healthy aging is of high relevance to this older population. The study was limited to White and Black participants. Strengths include the prospective design, population-based enrollment,

use of an objective marker of diet and metabolism, repeated measurements, and the rich information available on demographics, risk factors, and lifestyle habits.

## Conclusions

In conclusion, we report for the first time an association of higher levels of plasma phospholipid 24:0 and 22:0 with lower risk of incident unhealthy aging events. Together with our previous reports of inverse associations of VLSFA with incident heart failure,<sup>4</sup> incident atrial fibrillation,<sup>5</sup> incident sudden cardiac arrest,<sup>9</sup> and total mortality,<sup>10</sup> the study findings should prompt further research on determinants of VLSFA levels and may lead to novel approaches to promote healthy aging.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Abbreviations:

<b>VLSFA</b>	very-long-chain saturated fatty acid
<b>20:0</b>	arachidic acid
<b>22:0</b>	behenic acid
<b>24:0</b>	lignoceric acid
<b>CHS</b>	Cardiovascular Health Study
<b>BMI</b>	body mass index
<b>LDL</b>	low density lipoprotein
<b>HR</b>	hazard ratio

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### Key Points

**Question:**

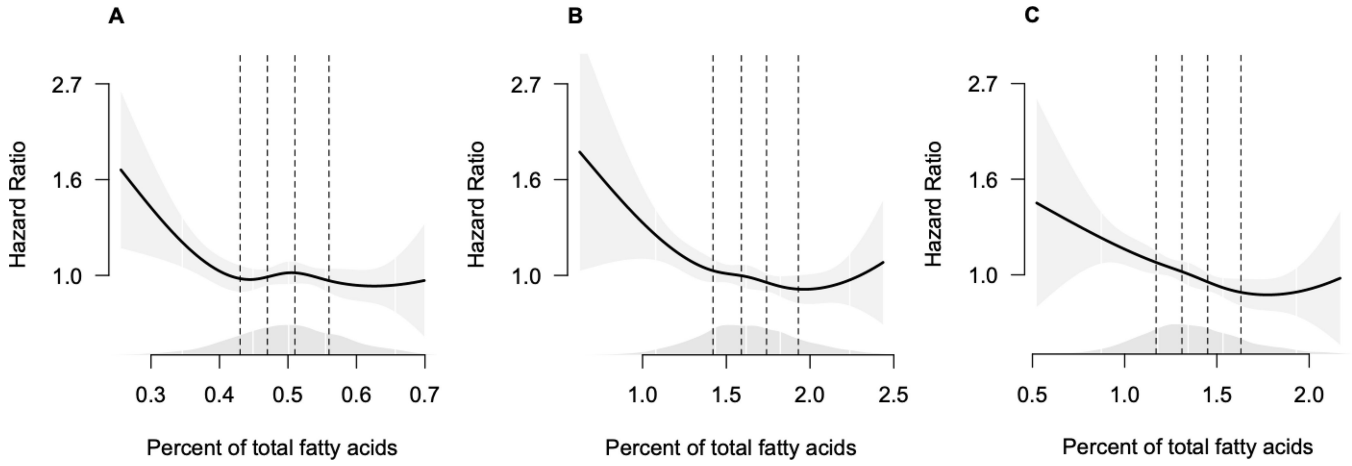
Are higher levels of circulating VLSFAs (20:0, 22:0 and 24:0) associated with healthy aging?

**Findings:**

In this large prospective cohort study of 2680 older individuals, participants in the highest quintile of levels of plasma phospholipid VLSFA 24:0 showed a significant 16% reduction in incident unhealthy aging events compared to those in the lowest quintile, with a similar finding for 22:0.

**Meaning:**

The study suggests that increasing circulating levels of VLSFA may be a novel target to promote healthy aging.



**Figure 1.** Hazard ratio (95% pointwise confidence interval) of unsuccessful aging associated with plasma phospholipid levels of VLSFAs relative to the median value  
 A = 20:0, B = 22:0, C = 24:0.  
 Each fatty acid was fit as a cubic spline with 4 knots. The presentation excludes the top 1% of values of each FA to illustrate the shape of the association with most of the data. Dashed lines indicate the quintile cutpoints. The shading around the line indicates the 95% pointwise confidence interval. Density plot for the fatty acid values shown just above the horizontal x-axis.  
 Models are adjusted for age, race (Black, non-Black), sex, field center, education (<HS, HS, Some college, College), physical activity (#blocks walked last week), BMI, waist circumference, alcohol consumption, smoking status (never, former, current), self-reported health ((Excellent/Very good, Good, Fair/Poor), prevalent diabetes, systolic blood pressure, use of hypertensive medications, and depression score.

Baseline characteristics of the Cardiovascular Health Study participants by quintiles of plasma phospholipid levels of 24:0

Table 1.

	Q1	Q2	Q3	Q4	Q5
Quintile cutpoints	<=1.17	>1.17-1.31	>1.31-1.45	>1.45-1.63	>1.63
Quintile median	1.07	1.25	1.38	1.53	1.78
Number of participants	546	532	530	540	532
<b>Participant characteristics:</b>					
Age, years	75.51 ±5.03	75.01 ±4.88	74.56 ±4.70	74.50 ±4.83	73.93 ±4.62
Male	146 (26.7%)	179 (33.6%)	190 (35.8%)	244 (45.2%)	217 (40.8%)
Black	47 (8.6%)	49 (9.2%)	54 (10.2%)	60 (11.1%)	98 (18.4%)
Field center					
NC	122 (22.3%)	126 (23.7%)	134 (25.3%)	140 (25.9%)	146 (27.4%)
CA	155 (28.4%)	125 (23.5%)	140 (26.4%)	144 (26.7%)	151 (28.4%)
MD	128 (23.4%)	127 (23.9%)	119 (22.5%)	108 (20.0%)	112 (21.1%)
PA	141 (25.8%)	154 (28.9%)	137 (25.8%)	148 (27.4%)	123 (23.1%)
Education					
<HS	107 (19.6%)	136 (25.6%)	122 (23.0%)	106 (19.6%)	102 (19.2%)
HS graduate	187 (34.2%)	165 (31.0%)	155 (29.2%)	140 (25.9%)	157 (29.5%)
Some coll.	134 (24.5%)	117 (22.0%)	123 (23.2%)	145 (26.9%)	115 (21.6%)
College grad.	118 (21.6%)	114 (21.4%)	130 (24.5%)	149 (27.6%)	158 (29.7%)
Body mass index (kg/m <sup>2</sup> )	27.15 ±4.81	26.84 ±4.65	26.31 ±4.27	26.44 ±4.12	26.12 ±4.00
Waist circumference (cm)	98.01 ±14.22	97.58 ±13.41	95.82 ±12.20	95.86 ±12.13	94.96 ±12.19
Systolic bp (mm hg)	138.94 ±21.59	135.67 ±20.18	135.00 ±19.33	132.99 ±20.26	134.17 ±21.55
LDL (mg/dl)	112.63 ±31.76	123.34 ±30.10	129.80 ±30.34	132.73 ±30.65	139.18 ±30.79
HDL (mg/dl)	53.75 ±15.89	53.92 ±14.19	54.13 ±13.83	54.17 ±14.12	55.48 ±13.15
Triglycerides (mg/dl)	192.32 ±121.45	147.61 ±72.07	132.62 ±61.97	123.53 ±54.57	107.91 ±46.45
C-reactive protein (mg/l)	5.95 ±10.67	4.67 ±7.65	4.20 ±8.54	4.28 ±8.81	3.91 ±5.81
Diabetes	143 (26.2%)	103 (19.4%)	86 (16.2%)	81 (15.0%)	91 (17.1%)
# Alcoholic beverages/wk	2.00 ±5.33	2.35 ±10.81	1.94 ±4.56	2.10 ±4.55	2.16 ±4.58
Blocks walked in previous week	37.80 ±62.56	48.47 ±80.71	47.37 ±66.42	54.29 ±74.58	47.75 ±69.76

	Q1	Q2	Q3	Q4	Q5
CES-D score	5.19 ±4.54	5.24 ±4.64	4.45 ±4.11	4.62 ±4.68	4.45 ±4.29
Anti-hypertensive medication	292 (53.5%)	242 (45.5%)	215 (40.6%)	208 (38.5%)	213 (40.0%)
Lipid-lowering medications	69 (12.6%)	44 (8.3%)	35 (6.6%)	32 (5.9%)	27 (5.1%)
Smoking status					
Never	299 (54.8%)	246 (46.2%)	274 (51.7%)	263 (48.7%)	250 (47.0%)
Former	211 (38.6%)	237 (44.5%)	206 (38.9%)	241 (44.6%)	230 (43.2%)
Current	36 (6.6%)	49 (9.2%)	50 (9.4%)	36 (6.7%)	52 (9.8%)
Self-reported health					
Excellent/VG	193 (35.3%)	221 (41.5%)	249 (47.0%)	282 (52.2%)	237 (44.5%)
Good	267 (48.9%)	238 (44.7%)	224 (42.3%)	206 (38.1%)	226 (42.5%)
Fair/Poor	86 (15.8%)	73 (13.7%)	57 (10.8%)	52 (9.6%)	69 (13.0%)
Dietary intake <sup>2</sup>					
Energy intake, kcal/day	1922.53 ±607.18	2002.80 ±612.90	2098.88 ±681.90	1956.53 ±591.68	2035.40 ±642.27
Protein, % energy	19.44 ±3.32	19.17 ±3.04	18.99 ±3.04	19.01 ±3.19	19.01 ±3.03
Carbohydrate, % energy	53.58 ±8.39	52.75 ±7.95	52.52 ±7.81	52.45 ±7.66	52.42 ±7.60
Total fat, % energy	30.77 ±6.16	31.58 ±6.12	32.11 ±5.98	32.20 ±5.92	32.30 ±5.92
Saturated fat, % energy	9.81 ±2.37	10.13 ±2.30	10.18 ±2.15	10.14 ±2.13	10.12 ±2.16
Polyunsaturated fat, % energy	7.12 ±2.11	7.23 ±2.19	7.51 ±2.15	7.64 ±2.31	7.70 ±2.12
Monounsaturated fat, % energy	11.01 ±2.53	11.37 ±2.49	11.55 ±2.40	11.54 ±2.36	11.65 ±2.39
Peanuts, servings/week	1.02 ±1.47	1.18 ±1.50	1.40 ±1.66	1.47 ±1.73	2.01 ±2.03

<sup>1</sup> Baseline is the time of the 1<sup>st</sup> VLSFA measurement

<sup>2</sup> Diet was assessed in 1989-90 and available only in participants enrolled in 1989-90.

**Table 2.**  
List of the Incident First Unhealthy Aging Events among 2680 CHS Participants

First incident unhealthy aging event	Number of events
Incident Cardiovascular disease <sup>a</sup>	880
Increase in difficulties of activities of daily living <sup>b</sup>	680
Incident Cancer	403
Cognitive decline <sup>c</sup>	376
Incident chronic obstructive pulmonary disease	133
Incident Kidney disease <sup>d</sup>	12
No event	196

<sup>a</sup> defined as myocardial infarction, heart failure, stroke, transient ischemic attack or claudication.

<sup>b</sup> first record of difficulty with 1 activity of daily living.

<sup>c</sup> first Mini Mental State Examination score < 80.

<sup>d</sup> defined as estimated glomerular filtration rate < 10 or dialysis.

**Table 3.**

Hazard ratio (95% confidence interval) of unsuccessful aging associated with higher quintiles of plasma phospholipid VLSFAs.

VLSFA	Q1	Q2	Q3	Q4	Q5	P trend	Per SD increment
<b>20:0</b>							
Events/P-Y	452/3662	434/3841	523/4461	522/4696	553/5152		
HR (95% CI)	1.00 (Ref.)	1.12 (0.98–1.27)	1.03 (0.90–1.17)	1.10 (0.98–1.25)	1.03 (0.91–1.16)	0.12	0.91 (0.81–1.00)
<b>22:0</b>							
Events/P-Y	563/4341	551/4562	495/4484	469/4298	406/4126		
HR (95% CI)	1.00 (Ref.)	0.93 (0.83–1.05)	0.89 (0.79–1.01)	0.90 (0.79–1.02)	0.85 (0.74–0.97)	0.01	0.87 (0.78–0.97)
<b>24:0</b>							
Events/P-Y	561/4278	530/4224	510/4597	462/4490	421/4223		
HR (95% CI)	1.00 (Ref.)	0.97 (0.86–1.10)	0.88 (0.78–1.00)	0.86 (0.76–0.97)	0.84 (0.73–0.95)	0.001	0.85 (0.77–0.94)

Models are adjusted for age, race (Black, non-Black), sex, field center, education (<HS, HS, Some college, College), physical activity (#blocks walked last week), BMI, waist circumference, alcohol consumption, smoking status (never, former, current), self-reported health ((Excellent/Very good, Good, Fair/Poor), prevalent diabetes, systolic blood pressure, use of hypertensive medications, and depression score.



**Table 4.**

Hazard ratio (95% confidence interval) of unsuccessful aging associated with higher quintiles of plasma phospholipid VLSFAs with further adjustment for the potential mediators triglycerides, LDL and 16:0

	Q1	Q2	Q3	Q4	Q5	p trend	Per std. increment
20:0	1.00 (Ref.)	1.08 (0.93–1.25)	1.01 (0.88–1.15)	1.09 (0.96–1.23)	1.02 (0.90–1.15)	0.36	0.93 (0.83–1.05)
22:0	1.00 (Ref.)	0.94 (0.83–1.07)	0.91 (0.80–1.04)	0.92 (0.80–1.06)	0.87 (0.75–1.02)	0.11	0.89 (0.78–1.02)
24:0	1.00 (Ref.)	0.97 (0.86–1.11)	0.89 (0.78–1.01)	0.87 (0.76–1.00)	0.85 (0.73–0.98)	0.007	0.86 (0.76–0.97)

Models are adjusted for age, race (Black, non-Black), sex, field center, education (<HS, HS, Some college, College), physical activity (#blocks walked last week), BMI, waist circumference, alcohol consumption, smoking status (never, former, current), self-reported health (Excellent/Very good, Good, Fair/Poor), systolic blood pressure, use of hypertensive medications, depression score, LDL, triglycerides, diabetes, and 16:0.