ORIGINAL RESEARCH

Serial Plasma Phospholipid Fatty Acids in the De Novo Lipogenesis Pathway and Total Mortality, Cause-Specific Mortality, and Cardiovascular Diseases in the Cardiovascular Health Study

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***Background*—**Synthesized fatty acids (FAs) from de novo lipogenesis may affect cardiometabolic health, but longitudinal associations between serially measured de novo lipogenesis–related fatty acid biomarkers and mortality or cardiovascular disease (CVD) are not well established.

***Methods and Results*—**We investigated longitudinal associations between de novo lipogenesis–related fatty acids with all-cause mortality, cause-specific mortality, and incident CVD among 3869 older US adults, mean (SD) age 75 (5) years and free of prevalent CVD at baseline. Levels of plasma phospholipid palmitic (16:0), palmitoleic (16:1n-7), stearic (18:0), oleic acid (18:1n-9), and other risk factors were serially measured at baseline, 6 years, and 13 years. All-cause mortality, cause-specific mortality, and incident fatal and nonfatal CVD were centrally adjudicated. Risk was assessed in multivariable-adjusted Cox models with time-varying FAs and covariates. During 13 years, median follow-up (maximum 22.4 years), participants experienced 3227 deaths (1131 CVD, 2096 non-CVD) and 1753 incident CVD events. After multivariable adjustment, higher cumulative levels of 16:0, 16:1n-7, and 18:1n-9 were associated with higher all-cause mortality, with extreme-quintile hazard ratios (95% CIs) of 1.35 (1.17–1.56), 1.40 (1.21–1.62), and 1.56 (1.35–1.80), respectively, whereas higher levels of 18:0 were associated with lower mortality (hazard ratio=0.76; 95% CI=0.66–0.88). Associations were generally similar for CVD mortality versus non-CVD mortality, as well as total incident CVD. Changes in levels of 16:0 were positively, and 18:0 inversely, associated with all-cause mortality (hazard ratio=1.23, 95% CI=1.08–1.41; and hazard ratio=0.78, 95% CI=0.68–0.90).

***Conclusions*—**Higher long-term levels of 16:0, 16:1n-7, and 18:1n-9 and changes in 16:0 were positively, whereas long-term levels and changes in 18:0 were inversely, associated with all-cause mortality in older adults. **(*J Am Heart Assoc*. 2019;8:e012881. DOI: 10.1161/JAHA.119.012881.)**

Key Words: cardiovascular disease • de novo lipogenesis • fatty acid biomarkers • longitudinal analysis • mortality

Hepatic de novo lipogenesis (DNL) is a regulated metabolic process wherein excess dietary starch, sugar, and protein are converted into specific fatty acids (FAs), in particular palmitic acid (16:0) and other saturated and monosaturated FAs.[1–4] Activation of DNL contributes to increased intrahepatic fat[5–7] and is associated with nonalcoholic fatty liver disease, insulin resistance,[6–8] atherogenic dyslipidemia,[8] and hypertriglyceridemia,[5,7,9] all risk factors for type 2 diabetes mellitus and cardiovascular disease (CVD).[5,6,10–14]

While DNL appears to influence risk of metabolic disease, direct measurement of DNL requires small-scale, costly isotope tracer studies.[5–8,15] This has limited the number and scope of investigations of DNL activity and long-term

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Accompanying Data S1, Tables S1 through S14, and Figures S1 through S13 are available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.119.012881

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Clinical Perspective

What Is New?

- De novo lipogenesis (DNL), the liver's process of turning dietary starch, sugar, and protein into fat, is increasingly linked to insulin resistance, diabetes mellitus, and other metabolic conditions; yet, how the fatty acid (FA) products of DNL relate to all-cause and cause-specific mortality remains less clear.
- We assessed how usual levels (measured serially over time) and changes in levels of DNL FA biomarkers measured in the blood, including palmitic acid (16:0), palmitoleic acid (16:1n-7), stearic acid (18:0), and oleic acid (18:1n-9), associate with death among older US adults through up to 22 years of follow-up.
- Higher usual-term levels of 16:0, 16:1n-7, and 18:1n-9 associated with higher risks of all-cause, CVD, and non-CVD mortality—higher levels of 18:0 associated with lower risk, and assessing changes in levels over time, changes in 16:0 positively associated, whereas changes in 18:0 inversely associated, with risk of death.

What Are the Clinical Implications?

- Higher long-term blood levels of specific FA related to DNL may confer greater risk of mortality and impair cardiometabolic health, suggesting that both DNL and these FAs could represent targets to reduce such risk.
- Observed protective associations for 18:0 are not consistent with limited previous studies, and further investigation of this FA is required.

health outcomes. FA profiling of objective FA metabolites of DNL provides an alternative estimation of DNL activity[12,16] as well as investigation of potential effects of individual FAs synthesized by DNL, which may have differing biologic actions. Major FAs in the DNL pathway include palmitic acid (16:0), palmitoleic acid (16:1n-7), stearic acid (18:0), and oleic acid (18:1n-9),[17,18] each of which appears to have significant bioactivity in animal and in vitro studies.[19–25]

In a randomized controlled trial, a reduction in dietary carbohydrates reduces hepatic steatosis among young nonalcoholic fatty liver disease patients, where the main proposed mechanism is a reduction in DNL activity.[26] Inversely, in human trials, carbohydrate-rich diets and alcohol intake increase DNL[7–9,27–31] as well as circulating levels of 16:0, 16:1n-7, and 18:1n-9[30,32,33] and, less consistently, 18:0.[33,34] Although these FAs are also directly consumed from the diet, correlations between dietary intakes of these FAs and their circulating levels tend to be weak.[35] In addition, a stepwise increase in carbohydrate intake and decrease in saturated fat intake leads to progressive increases in circulating levels of several of these FAs.[33] These findings suggest that circulating levels of these FAs are reasonable biomarkers of hepatic DNL.

In observational studies, associations between these FA biomarkers and major clinical outcomes are not well established. Higher levels of circulating 16:0 have been associated with higher risk of mortality[36] and diabetes mellitus,[35,37,38] whereas higher levels of 18:0 have been associated with lower risk of mortality but higher risk of diabetes mellitus.[35–38] Findings have been mixed or inconclusive for other major FAs in the DNL pathway in relation to risk of CVD[39] and CVD subtypes.[40–44] Furthermore, previous studies, including our own earlier work, have only evaluated a single measure of these FAs at baseline. Changes in DNL and these FA levels could result in misclassification over time, regression dilution bias, and attenuation toward the null. Serial measurements over many years allow assessment of both usual long-term levels as well as changes in levels over time, but relationships of serial measures of these FAs with major health outcomes have not been reported.

To address these gaps in knowledge, we used serial biomarkers of FAs in the DNL pathway, measured at 3 time points over 13 years, to investigate associations of long-term levels and changes in levels of these FAs with total mortality, cause-specific mortality, and incident total (fatal and nonfatal) CVD in the CHS (Cardiovascular Health Study). We hypothesized that a high level of circulating DNL FA, especially 16:0, would be associated with higher risk of all-cause mortality.

Methods

CHS data and study materials may be requested from the CHS Coordinating Center at https://chs-nhlbi.org/. Our study adhered to the reporting guidelines of the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement (Table S1).

Study Design and Population

The CHS is a multicenter, community-based, prospective cohort of older US adults.[45] In 1989–1990, 5201 noninstitutionalized adults aged >65 years who were not under active treatment for cancer were randomly selected and enrolled from Medicare eligibility lists in 4 US communities (Sacramento County, CA; Washington County, MD; Forsyth County, NC; and Pittsburgh, PA).[46] To increase minority representation, an additional 687 black participants were similarly recruited in 1992–1993. Among all eligible participants, 57% agreed to participate. Trained personnel performed annual study clinic examinations through 1999 to assess participant demographic characteristics, medical history, hospitalizations, and lifestyle through standardized protocols.[47,48] Semiannual

phone interviews were conducted continuously since enrollment to ascertain health status, incident, and mortality events through June 2015. In 2005–2006, remaining participants (n=1677) were evaluated in person or by phone to reassess medical history, hospitalizations, and lifestyle.[49] All protocols were approved at the institutional review board of each participating university. All participants also provided informed written consent.

Study Measures

Using stored plasma specimens collected at baseline (1992–1993, n=3941), 6 years (1998–1999, n=2609), and 13 years (2005–2006, n=933), levels of 46 distinct plasma phospholipid FAs were measured as a weighted percent of total FAs at the Fred Hutchinson Cancer Research Center Biomarker Laboratory. At each study collection, 12-hour fasting blood samples were collected and stored at −80°C, at which FA levels have been shown to be stable in long-term storage and multiple freeze-thaw cycles.[50] Compared with nonfasting phospholipid values, which may be influenced by the most recent meal, fasting phospholipid samples are more-stable biomarkers of usual dietary patterns over several months. Total lipids were extracted from plasma[51] and phospholipids separated from neutral lipids using 1-dimensional thin-layer chromatography.[52] FA methyl esters were derived from direct transesterification of phospholipid fractions[53] and separated by gas chromatography.[54] Identification, precision, and accuracy were evaluated throughout with model mixtures of known FA methyl esters and established in-house controls, with identification confirmed by gas chromatography/mass spectrometry at the US Department of Agriculture.[55] Laboratory coefficients of variation, assessed by a pooled plasma sample run together with each batch of study samples, were <3% for 16:0, 16:1n-7, 18:0, and 18:1n-9. Because these 10 816 FA assays were measured over different time periods, we evaluated the potential for laboratory drift among 163 CHS subjects in whom FAs were measured up to 15 years apart using the same stored samples. None of the measured FAs in the present analysis had evidence for appreciable lab drift (data not shown). After excluding the 592 participants who had died before 1992–1993 (baseline of this analysis), 737 without FA measures and 690 with prevalent CVD, a total of 3869 participants were included in this investigation.

Other Risk Factors

Sociodemographic information included age (years), sex (male, female), race (white, nonwhite), enrollment site, education (<high school, high school, some college, or college graduate), and income (<$12 000, $12 000–$24 999, $25 000–$49 999, or >$50 000/year). Other risk factors were assessed at the time of each FA measurement using standardized procedures, including anthropometrics (height and weight to calculate body mass index [in kg/m^2] and waist circumference [cm]), physical activity excluding chores (<500, 500–1000, 1000–1500, or >1500 kcal/week), blood pressure (mm Hg), high-density lipoprotein (mg/dL), low-density lipoprotein (mg/dL), triglycerides (mg/dL), and high-sensitivity C-reactive protein (in categories of <1, 1–3, and >3 mg/dL).[45,47,48,56,57] Information was collected on smoking status (nonsmoker, former smoker, or current smoker), self-perceived general health (excellent/very good, good, or fair/poor), family history of myocardial infarction and/or stroke (yes/no), incident diabetes mellitus (yes/no), hypertension medication (yes/no), and lipid medication (yes/no). Alcohol (wine, beer, and liquor; reported as 0, 0–0.5, 0.5–1, 1–2, 3–7, 8–14, and >14 servings/week) was assessed at each visit using validated questionnaires.[58] Dietary habits were assessed twice; in 1989–1990 using a 99-item validated food-frequency questionnaire[48] and in 1995–1996 using a 131-item self-administered validated food-frequency questionnaire,[59] including fruit intake (servings/day), vegetable intake (servings/day), processed meat intake (servings/day), total energy intake (kcal/day), and glycemic load. As expected given endogenous synthesis, correlations between circulating and dietary FAs in the DNL pathway were generally small, ranging between 0.01 and 0.21.[35] Other FA biomarkers, including omega-3 polyunsaturated fatty acids (n3-PUFAs; including the sum of α-linolenic acid, eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid), were quantified simultaneously with these FAs as weight percentage of total FAs, described above.

End Points

Our primary outcomes were all-cause mortality, CVD mortality (defined as death attributed to atherosclerotic CHD, cerebrovascular disease, other atherosclerotic disease, or other CVD), and total incident (fatal and nonfatal) CHD (defined as incident myocardial infarction or CHD death). Secondary outcomes included CVD subtypes (incident total CHD, stroke, ischemic stroke, and hemorrhagic stroke), non-CVD mortality (deaths attributed to cancer, pulmonary diseases, dementia, trauma/fracture, infection/sepsis, or other causes), and subtypes of non-CVD mortality. All outcomes were assessed and adjudicated by a centralized events committee using available data from interviews, next of kin, death certificates, hospitalizations, and other medical records, including diagnostics test and consultations. Algorithms and methods for follow-up, confirmation, and classification of deaths, CHD, and stroke have been described.[60,61] Vital status follow-up was 100% complete; <1% of all person-time was otherwise missing and censored early.

Statistical Analysis

Cox proportional hazards models were used to evaluate the association between time-varying DNL FA levels, adjusting for time-varying covariates, and outcomes. For all-cause mortality, there is no competing risk. For cause-specific deaths and incident fatal and nonfatal CVD, the Cox model accounts for competing risks by estimating associations with the cause-specific hazard function.[62] Time at risk was from the first FA measurement until first event, death, or the latest adjudicated date of follow-up in June 2015. There was little evidence that the proportional hazards assumption was violated[63] for all FAs except 18:1n-9, but proportional hazards was violated for some covariates. Thus, we adjusted for the covariates that violated proportional hazards using risk-set stratification, using a combined variable defined by these covariates; this stratified model no longer violated proportional hazards, except for quintiles 4 and 5 for 18:1n-9. Visual inspection of the Kaplan–Meier survival curve suggested this was attributable to similar and overlapping risks in these quintiles compared with quintiles 1 to 3 (Figure S1). When quintiles 3 to 5 were combined, proportional hazards assumption was met for 18:1n-9. Findings between the stratified models presented herein, and nonstratified models, did not differ meaningfully (data not shown).

To evaluate long-term (cumulative) exposure, time-varying FA levels were evaluated as weighted cumulative averages: At each time point, the average of current and past measurements was calculated, with 50% weight assigned to the most recent measure and equal weights for past measures.[64] For participants with missing FA levels (14.4% in 1998, 20.7% in 2005), the most recent measurement was carried forward. To assess changes in FA levels, the mean percent change in FA levels was evaluated among participants with $\geq$2 measurements (n=1815). Percent change in FA levels from 1992 to 1998 were related to risk between 1998 and 2005, and mean percent change in FA levels for 1992–1998 and 1998–2005 combined were related to risk between 2005 and 2015. Exposures were evaluated categorically in quintiles as indicator variables, with quintile cut points based on study baseline measures. To assess the significance of trends across quintiles, quintiles were assessed as continuous variables after assigning participants the median value in each quintile.[64] FA levels were also evaluated continuously per interquintile range, the difference between the midpoint (median) value of the first and fifth quintiles. The analysis using indicator quintiles makes no assumptions about linearity and also minimizes the effects of outliers. The complementary interquintile range analysis tests a potential linear relationship with maximum statistical power, although with stronger assumptions about linearity. Potential nonlinear associations were explored semiparametrically using restricted cubic splines.[65]

Covariates were selected based on biological interest, current, or previously observed associations with these FAs or mortality and meaningful changes in the exposure relative risk estimate ($\pm$5%). Missing covariates were imputed by best-subset regression at each time point (range of missingness: 0.1–6.5% in 1992–1993, 2.0–21.1% in 1998–1999, and 7.5–42.2% in 2005–2006) using 17 demographic and lifestyle variables (plus up to 4 additional dietary variables for missing dietary factors). Findings were similar when participants with missing values were excluded (data not shown).

In sensitivity analysis, we excluded participants with poor self-reported health and additionally adjusted for dietary factors and plasma phospholipid n3-PUFAs. De novo lipogenesis is a regulated metabolic process that converts excess dietary starch and sugar into saturated and monosaturated FAs. Increased levels of these DNL-related FAs are associated with hepatic steatosis, which is in turn linked to insulin resistance, high blood pressure, dyslipidemia, and type 2 diabetes mellitus. Thus, such risk factors could be in the downstream pathway (mediators) between DNL FAs and clinical events; and adjustment for these factors could represent overadjustment. Thus, we adjusted for lipid medication use, incident diabetes mellitus, triglyceride levels, and C-reactive protein levels in sensitivity analyses as potential mediators. We also additionally adjusted for 16:0 levels when evaluating associations between 18:0 levels and mortality to consider its independent effects. In secondary analyses, we evaluated other FAs that are minor products of DNL, including myristic acid (14:0; coefficients of variation<8%), 7-hexadecenoic acid (16:1n-9; coefficients of variation<8%), and vaccenic acid (18:1n-7; coefficients of variation<3%). Interaction was explored by age, sex, body mass index, waist circumference, diabetes mellitus, and self-reported health by including multiplicative interaction terms with each FA, corrected for multiple comparisons at Bonferroni 2-tailed α<0.001 (4 major and 3 minor FAs$\times$6 interaction factors=42 exploratory comparisons). We did not adjust for multiple comparisons, given prespecified hypotheses for major DNL FAs and our primary outcomes (all-cause mortality, CVD mortality, and incident CVD), but exercised caution when interpreting results unrelated to the primary hypotheses; paid close attention to internal consistency and findings of others; and gave appropriate weight in interpretation to biological plausibility based on known pathophysiology, biochemistry, and molecular genetics. Statistical significance of the hazard ratio (HR) for each clinical end point was defined as 2-tailed α=0.05. Analyses were performed using Stata software (release 14.2; StataCorp LP, College Station, TX).[66]

Results

Participant Characteristics

At baseline in 1992–1993, mean (SD) age was 75 (5) years, 62% were female, and 12% were nonwhite (Table). Educational

Table. Baseline Characteristics of 3333 Participants in 1992–1993*

Variables†	Mean (SD) or n (%)
Demographics	
Age, mean (SD), y	75.0 (5.2)
Female, n (%)	2075 (62.3)
Race	
White, n (%)	2922 (87.7)
Nonwhite, n (%)	411 (12.3)
Education, n (%)	
High school	859 (25.8)
High school	947 (28.4)
Some college	781 (23.4)
College graduate	746 (22.4)
Annual income group, n (%)	
<$11 999	773 (23.2)
$12 000 to $24 999	1166 (35.0)
$25 000 to $49 999	926 (27.8)
>$50 000	468 (14.0)
Enrollment site, n (%)	
Bowman Gray	867 (26.0)
Davis	866 (26.0)
Hopkins	757 (22.7)
Pittsburgh	843 (25.3)
Lifestyle	
Health status (self-report), n (%)	
Excellent/very good	1464 (43.9)
Good	1233 (37.0)
Fair/poor	636 (19.1)
Smoking, n (%)	
Current smokers	351 (10.5)
Former smokers	1366 (41.0)
Never smokers	1616 (48.5)
Physical activity, mean (SD), mcal/week	1.2 (1.4)
Alcohol, mean (SD), servings/week	2.6 (6.4)
BMI, mean (SD), kg/m^2	26.7 (4.7)
Waist circumference, mean (SD), cm	97.0 (13.3)
Medical history	
Lipid medication, n (%)	149 (4.5)
Hypertension medication, n (%)	1362 (40.9)
Prevalent diabetes mellitus, n (%)	366 (11.0)
Family history of myocardial infarction or stroke, n (%)	965 (29.0)
Fatty acid biomarkers (% total fatty acids)	
Palmitic acid (16:0), median (range)	25.3 (19.5–33.1)
Palmitoleic acid (16:1n-7), median (range)	0.45 (0.11–1.87)
Stearic acid (18:0), median (range)	13.4 (8.2–18.9)
Oleic acid (18:1n-9), median (range)	7.5 (4.9–14.2)

*Characteristics reported here are for the 3333 participants who entered the analysis at baseline. Another 508 entered at the time of their first fatty acid measurement in 1998–1999 (year 6), and 28 entered in 2005–2006 (year 13), equating to a total of 3869 participants in the analysis.
†Values reported as mean (SD) for continuous variables, and frequency, (percent) for categorical variables, unless otherwise stated.

attainment ranged from <high school (26%) to college graduates (22%). Nearly half were never smokers (49%) and 81% self-reported being in good or very good/excellent health. Mean (SD) body mass index and waist circumference were 27 (5) kg/m^2 and 97 (13) cm, respectively; and alcohol intake, 2.6 (6.4) servings/week. Around 1 in 10 participants had prevalent diabetes mellitus, whereas 4 in 10 were taking antihypertensive medications. Median levels of these FAs in the DNL pathway ranged from 0.45% to 25.3%, with highest levels for 16:0.

Basic demographics (age, sex, race, education, income, and enrollment site) were similar across quintiles of the 4 FAs (Tables S2 through S5). However, different patterns were observed for physical activity, alcohol consumption, lipid biomarkers, inflammatory markers, other FA biomarkers, and dietary habits. For example, participants with higher levels of 16:0 were more likely to be physically active, have higher alcohol intake, and less likely to have a family history of CVD, whereas opposing patterns were observed for 18:0. Characteristics of participants who died before FA measurement or who were alive and missing FA measurements were largely similar to those included in the analysis, except those who died were slightly older and slightly more likely to be male, white, less educated, have a lower income, consume less alcohol, and report fair/poor health status, whereas those with missing FA measurements were slightly more likely to be less educated and have lower income (Table S6). As expected, participants who died during follow-up were also more likely to be older, male, white, less educated, have a lower income, be a current smoker, exercise less, and report fair/poor health status (Table S7).

Mean cohort levels of each FA across 13 years of serial measures are shown in Figure S2. Spearman correlations for serial levels of each FA, reflecting reproducibility over time, ranged from 0.40 to 0.67 (Table S8). Pairwise correlations between 16:0, 16:1n-7, and 18:1n-9 were generally low to modest (r=0.15–0.57) and negatively correlated to 18:0

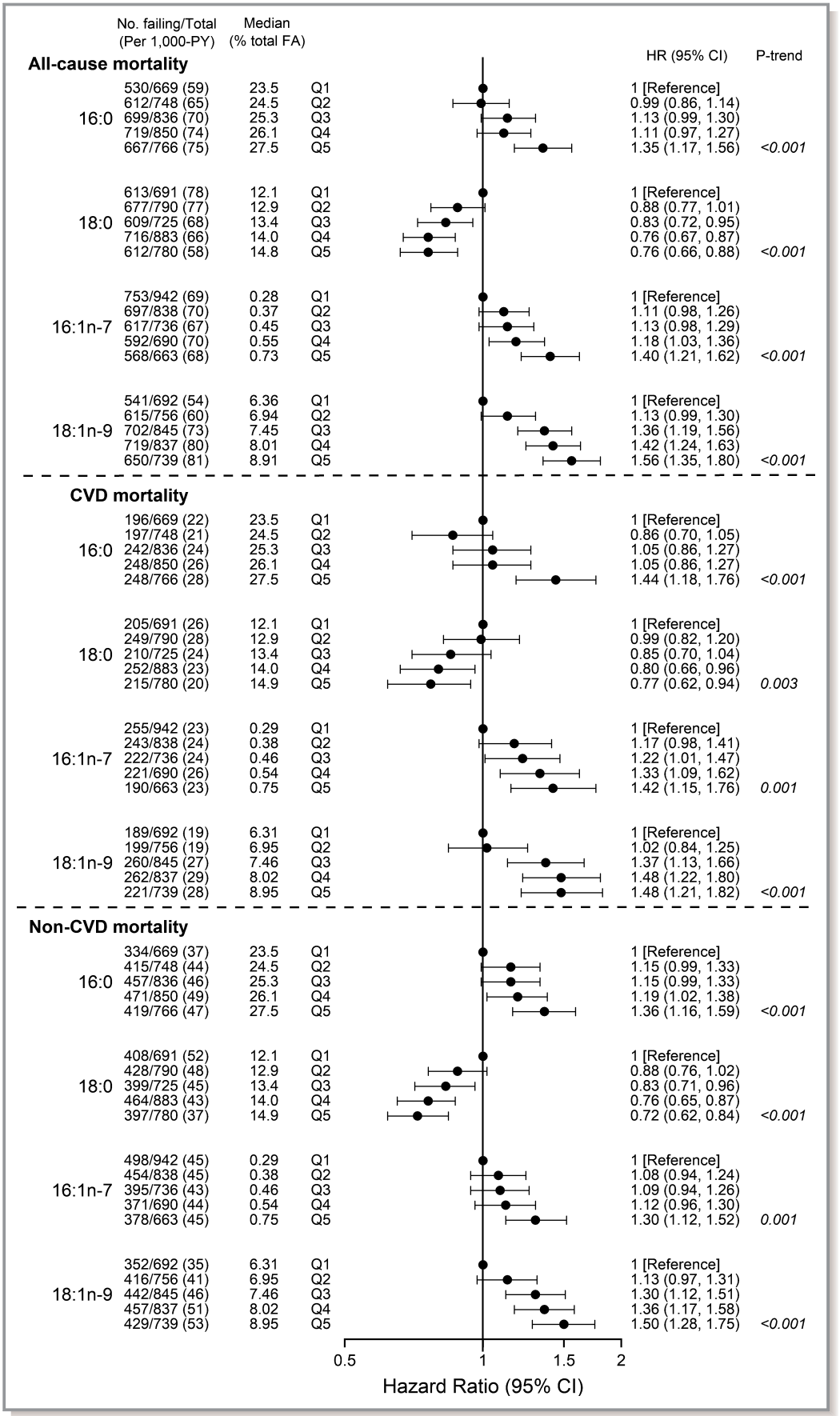
No. failing/Total (Per 1,000-PY)
Median (% total FA)
HR (95% CI)
P-trend
All-cause mortality
16:0
530/669 (59) 23.5 Q1 1 [Reference]
612/748 (65) 24.5 Q2 0.99 (0.86, 1.14)
699/836 (70) 25.3 Q3 1.13 (0.99, 1.30)
719/850 (74) 26.1 Q4 1.11 (0.97, 1.27)
667/766 (75) 27.5 Q5 1.35 (1.17, 1.56) <0.001
18:0
613/691 (78) 12.1 Q1 1 [Reference]
677/790 (77) 12.9 Q2 0.88 (0.77, 1.01)
609/725 (68) 13.4 Q3 0.83 (0.72, 0.95)
716/883 (66) 14.0 Q4 0.76 (0.67, 0.87)
612/780 (58) 14.8 Q5 0.76 (0.66, 0.88) <0.001
16:1n-7
753/942 (69) 0.28 Q1 1 [Reference]
697/838 (70) 0.37 Q2 1.11 (0.98, 1.26)
617/736 (67) 0.45 Q3 1.13 (0.98, 1.29)
592/690 (70) 0.55 Q4 1.18 (1.03, 1.36)
568/663 (68) 0.73 Q5 1.40 (1.21, 1.62) <0.001
18:1n-9
541/692 (54) 6.36 Q1 1 [Reference]
615/756 (60) 6.94 Q2 1.13 (0.99, 1.30)
702/845 (73) 7.45 Q3 1.36 (1.19, 1.56)
719/837 (80) 8.01 Q4 1.42 (1.24, 1.63)
650/739 (81) 8.91 Q5 1.56 (1.35, 1.80) <0.001
CVD mortality
16:0
196/669 (22) 23.5 Q1 1 [Reference]
197/748 (21) 24.5 Q2 0.86 (0.70, 1.05)
242/836 (24) 25.3 Q3 1.05 (0.86, 1.27)
248/850 (26) 26.1 Q4 1.05 (0.86, 1.27)
248/766 (28) 27.5 Q5 1.44 (1.18, 1.76) <0.001
18:0
205/691 (26) 12.1 Q1 1 [Reference]
249/790 (28) 12.9 Q2 0.99 (0.82, 1.20)
210/725 (24) 13.4 Q3 0.85 (0.70, 1.04)
252/883 (23) 14.0 Q4 0.80 (0.66, 0.96)
215/780 (20) 14.9 Q5 0.77 (0.62, 0.94) 0.003
16:1n-7
255/942 (23) 0.29 Q1 1 [Reference]
243/838 (24) 0.38 Q2 1.17 (0.98, 1.41)
222/736 (24) 0.46 Q3 1.22 (1.01, 1.47)
221/690 (26) 0.54 Q4 1.33 (1.09, 1.62)
190/663 (23) 0.75 Q5 1.42 (1.15, 1.76) 0.001
18:1n-9
189/692 (19) 6.31 Q1 1 [Reference]
199/756 (19) 6.95 Q2 1.02 (0.84, 1.25)
260/845 (27) 7.46 Q3 1.37 (1.13, 1.66)
262/837 (29) 8.02 Q4 1.48 (1.22, 1.80)
221/739 (28) 8.95 Q5 1.48 (1.21, 1.82) <0.001
Non-CVD mortality
16:0
334/669 (37) 23.5 Q1 1 [Reference]
415/748 (44) 24.5 Q2 1.15 (0.99, 1.33)
457/836 (46) 25.3 Q3 1.15 (0.99, 1.33)
471/850 (49) 26.1 Q4 1.19 (1.02, 1.38)
419/766 (47) 27.5 Q5 1.36 (1.16, 1.59) <0.001
18:0
408/691 (52) 12.1 Q1 1 [Reference]
428/790 (48) 12.9 Q2 0.88 (0.76, 1.02)
399/725 (45) 13.4 Q3 0.83 (0.71, 0.96)
464/883 (43) 14.0 Q4 0.76 (0.65, 0.87)
397/780 (37) 14.9 Q5 0.72 (0.62, 0.84) <0.001
16:1n-7
498/942 (45) 0.29 Q1 1 [Reference]
454/838 (45) 0.38 Q2 1.08 (0.94, 1.24)
395/736 (43) 0.46 Q3 1.09 (0.94, 1.26)
371/690 (44) 0.54 Q4 1.12 (0.96, 1.30)
378/663 (45) 0.75 Q5 1.30 (1.12, 1.52) 0.001
18:1n-9
352/692 (35) 6.31 Q1 1 [Reference]
416/756 (41) 6.95 Q2 1.13 (0.97, 1.31)
442/845 (46) 7.46 Q3 1.30 (1.12, 1.51)
457/837 (51) 8.02 Q4 1.36 (1.17, 1.58)
429/739 (53) 8.95 Q5 1.50 (1.28, 1.75) <0.001
0.5
1
1.5
2
Hazard Ratio (95% CI)

Figure 1. Major fatty acids from the de novo lipogenesis pathway and the risk of all-cause mortality, cardiovascular mortality, and noncardiovascular mortality in the Cardiovascular Health Study after 22 years of maximum follow-up among 3869 older adults. *P* trend was calculated by assessing quintiles as continuous variables after assigning participants the median value in each quintile. Multivariable adjustments include age (years), sex (male, female), race (white, nonwhite), enrollment site (Bowman Gray, Davis, Hopkins, or Pittsburgh), education (<high school, high school, some college, or college graduate), income (<$11 999, $12 000-$24 999, $25 000–$49 999, or >$50 000/year), body mass index (kg/m^2), physical activity (<500, 500–1000, 1000–1500, or >1500 kcal/week), waist circumference (cm), alcohol intake (0, 0–0.5, 0.5–1, 1–2, 3–7, 8–14, or >14 servings/week), smoking (nonsmokers, former smokers, and current smokers), self-reported health (excellent/very good, good, or fair/poor), and family history of cardiovascular disease (yes, no). CVD indicates cardiovascular disease; FA, fatty acid; HR, hazard ratio; PY, person-years; 16:0, palmitic acid; 16:1n-7, palmitoleic acid; 18:0, stearic acid; 18:1n-9, oleic acid.

(r=−0.08 to −0.45). Partial correlations adjusted for age and sex were evaluated between 16:0, 16:1n-7, 18:0, and 18:1n-9 and CVD risk factors (including low-density lipoprotein-cholesterol, high-density lipoprotein-cholesterol, triglycerides, systolic blood pressure, and fasting glucose). Correlations were also generally low (Table S9), with the strongest correlations between 16:0, 16:1n-7, and triglycerides (r=0.12–0.23), supporting a relationship of these FAs with DNL given that triglycerides are produced downstream in the DNL pathway.

Total and Cause-Specific Mortality

During 46 974 person-years of follow-up, participants experienced 3227 deaths (1131 from CVD and 2096 from non-CVD causes) and 1753 total incident (fatal and nonfatal) CVD events.

After multivariable adjustment, including for demographics, lifestyle, cardiometabolic risks, dietary habits, and other FAs, higher long-term (cumulative) levels of 16:0, 16:1n-7, and 18:1n-9 were positively associated with all-cause, total CVD, and non-CVD mortality, whereas 18:0 was inversely associated with the risk of these same outcomes (Figure 1). Participants in the highest quintile of 16:0, 16:1n-7, and 18:1n-9 had a 35% to 56% higher risk of all-cause mortality, 42% to 48% higher risk of total CVD mortality, and 30% to 50% higher risk of non-CVD mortality, compared with the lowest quintile of each FA. In contrast, participants in the highest quintile for 18:0 had lower risk of all-cause, total CVD, and non-CVD mortality with risk reductions between 23% and 28%, compared with the lowest quintile (*P* for trend≤0.003 for all). Unadjusted analyses are presented in Table S10.

Incident Total CVD

Similar to findings for total mortality, long-term levels of 16:0, 16:1n-7, and 18:1n-9 were each positively associated with incident total CVD, whereas 18:0 was inversely associated (Figure 2). Unadjusted analyses are presented in Table S11. In secondary analyses of CVD subtypes, findings were stronger for incident total stroke (Figure 2); results for stroke subtypes were generally not statistically significant (Figure S3).

Continuous Linear Assessment

Findings were similar when each FA was evaluated in linear models. Per interquintile range, 16:0, 16:1n-7, and 18:1n-9 were each positively associated with all-cause mortality (Figure S4) and incident CVD (Figure S5), whereas 18:0 was inversely associated. In secondary analyses of CVD subtypes, 16:0, 16:1n-7, and 18:1n-9 were positively, and 18:0 inversely, associated with incident stroke, whereas 18:1n-9 was also associated with higher risk of incident CHD.

For most associations, restricted cubic splines did not reveal statistically significant departure from linearity (Figure 3), although possible threshold effects were observed for 18:0 and 18:1n-9.

Changes in FA Levels Over Time

For assessment of changes in FA levels, time at risk began at the time of the second FA measurement, resulting in fewer participants, less follow-up-time, and fewer events compared with analyses of long-term cumulative FA levels. Nonetheless, findings for mortality were generally consistent with results for long-term levels: Changes in 16:0 and 18:1n-9 levels were positively associated with higher risk of all-cause mortality, CVD mortality, and non-CVD mortality, whereas changes in 18:0 levels were inversely associated with lower risk of all-cause mortality and non-CVD mortality (Figure 4). Associations of changes in levels of these FAs with incident total CVD or CVD subtypes generally did not achieve statistical significance (Figure S6).

Sensitivity Analyses

Findings for long-term levels and changes in levels of these FAs and total mortality were not appreciably altered after excluding participants with poor self-reported health or without carrying forward past measurements if missing

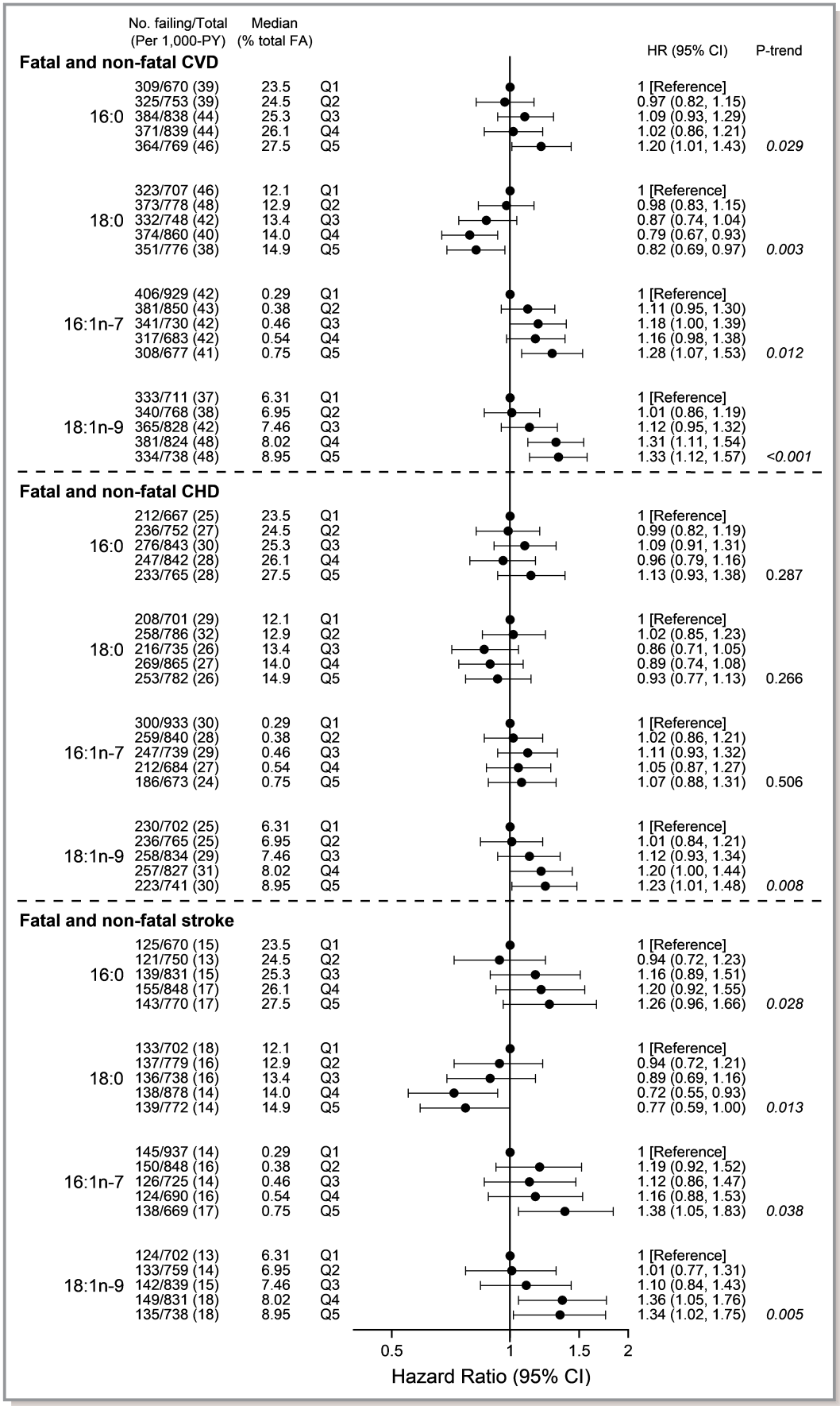
No. failing/Total (Per 1,000-PY)
Median (% total FA)
HR (95% CI)
P-trend
Fatal and non-fatal CVD
16:0
309/670 (39) 23.5 Q1 1 [Reference]
325/753 (39) 24.5 Q2 0.97 (0.82, 1.15)
384/838 (44) 25.3 Q3 1.09 (0.93, 1.29)
371/839 (44) 26.1 Q4 1.02 (0.86, 1.21)
364/769 (46) 27.5 Q5 1.20 (1.01, 1.43) 0.029
18:0
323/707 (46) 12.1 Q1 1 [Reference]
373/778 (48) 12.9 Q2 0.98 (0.83, 1.15)
332/748 (42) 13.4 Q3 0.87 (0.74, 1.04)
374/860 (40) 14.0 Q4 0.79 (0.67, 0.93)
351/776 (38) 14.9 Q5 0.82 (0.69, 0.97) 0.003
16:1n-7
406/929 (42) 0.29 Q1 1 [Reference]
381/850 (43) 0.38 Q2 1.11 (0.95, 1.30)
341/730 (42) 0.46 Q3 1.18 (1.00, 1.39)
317/683 (42) 0.54 Q4 1.16 (0.98, 1.38)
308/677 (41) 0.75 Q5 1.28 (1.07, 1.53) 0.012
18:1n-9
333/711 (37) 6.31 Q1 1 [Reference]
340/768 (38) 6.95 Q2 1.01 (0.86, 1.19)
365/828 (42) 7.46 Q3 1.12 (0.95, 1.32)
381/824 (48) 8.02 Q4 1.31 (1.11, 1.54)
334/738 (48) 8.95 Q5 1.33 (1.12, 1.57) <0.001
Fatal and non-fatal CHD
16:0
212/667 (25) 23.5 Q1 1 [Reference]
236/752 (27) 24.5 Q2 0.99 (0.82, 1.19)
276/843 (30) 25.3 Q3 1.09 (0.91, 1.31)
247/842 (28) 26.1 Q4 0.96 (0.79, 1.16)
233/765 (28) 27.5 Q5 1.13 (0.93, 1.38) 0.287
18:0
208/701 (29) 12.1 Q1 1 [Reference]
258/786 (32) 12.9 Q2 1.02 (0.85, 1.23)
216/735 (26) 13.4 Q3 0.86 (0.71, 1.05)
269/865 (27) 14.0 Q4 0.89 (0.74, 1.08)
253/782 (26) 14.9 Q5 0.93 (0.77, 1.13) 0.266
16:1n-7
300/933 (30) 0.29 Q1 1 [Reference]
259/840 (28) 0.38 Q2 1.02 (0.86, 1.21)
247/739 (29) 0.46 Q3 1.11 (0.93, 1.32)
212/684 (27) 0.54 Q4 1.05 (0.87, 1.27)
186/673 (24) 0.75 Q5 1.07 (0.88, 1.31) 0.506
18:1n-9
230/702 (25) 6.31 Q1 1 [Reference]
236/765 (25) 6.95 Q2 1.01 (0.84, 1.21)
258/834 (29) 7.46 Q3 1.12 (0.93, 1.34)
257/827 (31) 8.02 Q4 1.20 (1.00, 1.44)
223/741 (30) 8.95 Q5 1.23 (1.01, 1.48) 0.008
Fatal and non-fatal stroke
16:0
125/670 (15) 23.5 Q1 1 [Reference]
121/750 (13) 24.5 Q2 0.94 (0.72, 1.23)
139/831 (15) 25.3 Q3 1.16 (0.89, 1.51)
155/848 (17) 26.1 Q4 1.20 (0.92, 1.55)
143/770 (17) 27.5 Q5 1.26 (0.96, 1.66) 0.028
18:0
133/702 (18) 12.1 Q1 1 [Reference]
137/779 (16) 12.9 Q2 0.94 (0.72, 1.21)
136/738 (16) 13.4 Q3 0.89 (0.69, 1.16)
138/878 (14) 14.0 Q4 0.72 (0.55, 0.93)
139/772 (14) 14.9 Q5 0.77 (0.59, 1.00) 0.013
16:1n-7
145/937 (14) 0.29 Q1 1 [Reference]
150/848 (16) 0.38 Q2 1.19 (0.92, 1.52)
126/725 (14) 0.46 Q3 1.12 (0.86, 1.47)
124/690 (16) 0.54 Q4 1.16 (0.88, 1.53)
138/669 (17) 0.75 Q5 1.38 (1.05, 1.83) 0.038
18:1n-9
124/702 (13) 6.31 Q1 1 [Reference]
133/759 (14) 6.95 Q2 1.01 (0.77, 1.31)
142/839 (15) 7.46 Q3 1.10 (0.84, 1.43)
149/831 (18) 8.02 Q4 1.36 (1.05, 1.76)
135/738 (18) 8.95 Q5 1.34 (1.02, 1.75) 0.005
0.5
1
1.5
2
Hazard Ratio (95% CI)

Figure 2. Major fatty acids from the de novo lipogenesis pathway and the risk of fatal and nonfatal cardiovascular disease, coronary heart disease (CHD), and stroke in the Cardiovascular Health Study after 22 years of maximum follow-up among 3869 older adults. *P* trend was calculated by assessing quintiles as continuous variables after assigning participants the median value in each quintile. Multivariable adjustments include age (years), sex (male, female), race (white, nonwhite), enrollment site (Bowman Gray, Davis, Hopkins, or Pittsburgh), education (<high school, high school, some college, or college graduate), income (<$11 999, $12 000–$24 999, $25 000–$49 999, or >$50 000/year), body mass index (kg/m^2), physical activity (<500, 500–1000, 1000–1500, or >1500 kcal/week), waist circumference (cm), alcohol intake (0, 0–0.5, 0.5–1, 1–2, 3–7, 8–14, or >14 servings/week), smoking (nonsmokers, former smokers, and current smokers), self-reported health (excellent/very good, good, or fair/poor), and family history of cardiovascular disease (yes, no). CHD indicates coronary heart disease; CVD, cardiovascular disease; FA, fatty acid; HR, hazard ratio; PY, person-years; 16:0, palmitic acid; 16:1n-7, palmitoleic acid; 18:0, stearic acid; 18:1n-9, oleic acid.

(data not shown). Findings also remained similar following adjustment for dietary factors and plasma phospholipid n3-PUFAs. However, some associations were attenuated with adjustment for potential mediators, including lipid-lowering medication use, prevalent diabetes mellitus, triglyceride levels, and C-reactive protein levels. For example, in models adjusting for potential mediators, relationships were attenuated for 16:0, 16:1n-7, and 18:0 and all-cause mortality (per interquintile range, HR [95% CI] 1.16 [0.92–1.45], 1.13 [0.92–1.39], and 0.85 [0.68–1.06]). Associations were also attenuated for 16:0 and 16:1n-7 and incident total CVD (1.09 [0.94–1.26]; 1.06 [0.93–1.20]) and for 16:1n-7 and stroke incidence (1.20 [0.98–1.47]). When 16:0 was additionally adjusted for in models evaluating the other FAs, inverse associations between 18:0 and all-cause mortality and non-CVD mortality were attenuated, whereas findings for 18:0 and incident CVD as well as other FAs and mortality and CVD outcomes were not appreciably altered (Figure S7).

Findings were mixed and inconsistent for other, minor FAs in the DNL pathway (Figures S4 and S5, S8 through S11, and Table S12).

Exploratory Analyses

Exploratory findings for subtypes of non-CVD mortality are presented in Data S1 (Figures S12 and S13).

There was little evidence that associations of DNL-related FAs with mortality varied by sex, body mass index, waist circumference, or self-reported health (Tables S13 and S14).

Discussion

In this prospective cohort of community-based older US adults, higher long-term levels of 16:0, 16:1n-7, and 18:1n-9 and changes over time in 16:0 were positively associated with all-cause mortality, whereas long-term levels and changes over time in 18:0 were inversely associated with all-cause mortality. In general, risk was 30% to 49% higher across quintiles of 16:0, 16:1n-7, and 18:1n-9, whereas risk was 29% lower across quintiles of 18:0. Associations were similar for CVD death and non-CVD death, as well as for total incident CVD, and were robust to several sensitivity analyses. Associations also appeared generally linear. To our knowledge, this is the first investigation to assess the relationship between serial measures of major FA biomarkers in the DNL pathway and mortality.

Mechanistic studies support potential harms of circulating or tissue levels of 16:0, 16:1n-7, and 18:1n-9. Observed effects in experimental studies include increases in inflammation,[21,23,25] effects on gene expression in key pathways associated with inflammation, glucose metabolism, and lipogenesis (peroxisome proliferator-activated receptor gamma, peroxisome proliferator-activated receptor alpha, sterol regulatory element-binding transcription factor 1, nuclear factor kappa-light-chain-enhancer of activated B cells, monocyte chemoattractant protein-1, interleukin-6, and cyclooxygenase-2),[19,24] increased endoplasmic reticulum stress,[20,23] apoptosis in multiple cell types,[19,20,22] induction of cytotoxic steatosis,[19,22] and β-cell dysfunction.[21] Together, these mechanisms are related to nonalcoholic fatty liver disease, type 2 diabetes mellitus, cancer, and CVD and therefore support the biological plausibility of our results. However, the similar observed risk for both CVD and non-CVD mortality may suggest a larger role for underlying biological mechanisms that are more common to diseases of aging in general, such as inflammation and apoptosis, rather than highly disease-specific mechanisms.

Several determinants influence the levels of these FAs in the body. Direct sources of 16:0 and 18:1n-9 are abundant in the diet, but dietary intakes of these FAs appear to be poorly correlated with circulating levels.[35,44] These FAs are also endogenous products of the DNL pathway, especially for 16:1n-7.[44] Key substrates for their synthesis in the liver include excess dietary carbohydrates, especially rapidly digesting refined starch and sugar, and alcohol,[31,33] which enhance the rate of DNL and the production of these circulating FAs. Dietary

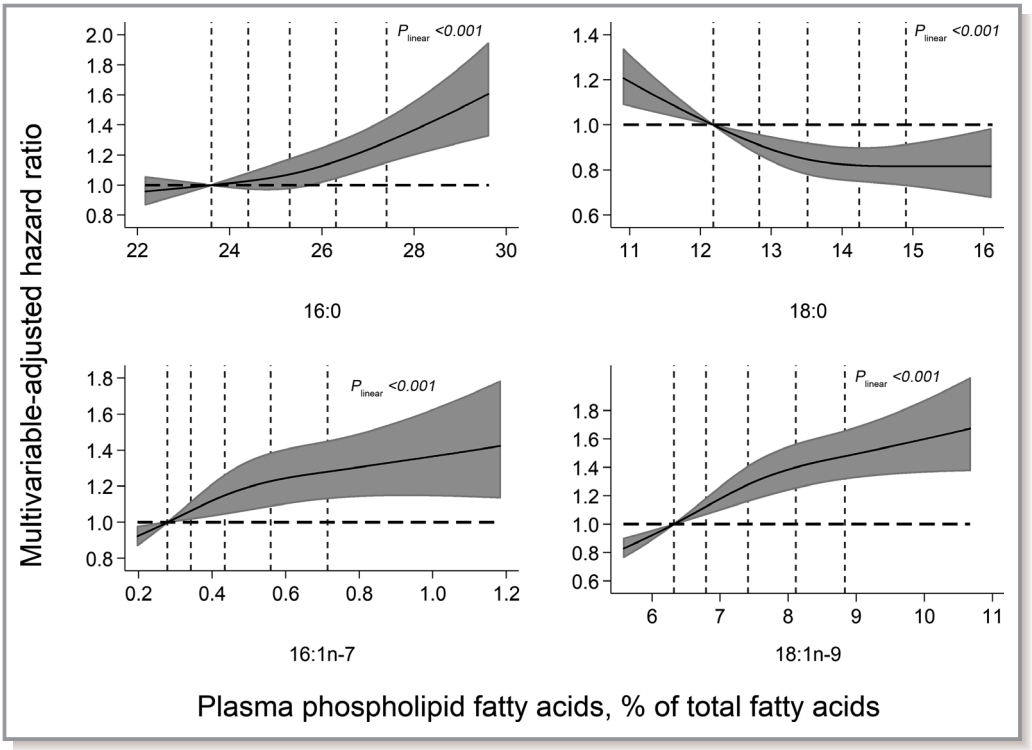

Figure 3. Multivariable-adjusted relationship of major cumulative plasma phospholipid fatty acids from the de novo lipogenesis pathway with risk of all-cause mortality, evaluated using restricted cubic splines. The solid lines and shaded area represent the central risk estimate and 95% CI, respectively, for each fatty acid. The dotted vertical lines correspond to the 10th, 25th, 50th, 75th, and 90th percentiles for each fatty acid. The top and bottom 1% of participants were omitted as outliers to provide better visualization. Evidence for nonlinearity (P_{curve}) was calculated by performing a likelihood ratio test between a multivariable model with all spline terms vs a multivariable model with only the linear term, whereas evidence for (P_{linear}) was calculated by performing a likelihood ratio test between a multivariable model without spline terms vs a multivariable model with only the linear term. No evidence for nonlinearity was found for 16:0 and 16:1n-7, where P_{curve}=0.19 and P_{curve}=0.11; for 18:0 and 18:1n-9, P_{curve}=0.04 and P_{curve}=0.02, suggesting a possible threshold effect. Multivariable adjustments include age (years), sex (male, female), race (white, nonwhite), enrollment site (Bowman Gray, Davis, Hopkins, or Pittsburgh), education (<high school, high school, some college, or college graduate), income (<$11 999, $12 000–$24 999, $25 000–$49 999, or >$50 000/year), body mass index (kg/m^2), physical activity (<500, 500–1000, 1000–1500, or >1500 kcal/week), waist circumference (cm), alcohol intake (0, 0–0.5, 0.5–1, 1–2, 3–7, 8–14, or >14 servings/week), smoking (nonsmokers, former smokers, and current smokers), self-reported health (excellent/very good, good, or fair/poor), and family history of cardiovascular disease (yes, no). 16:0 indicates palmitic acid; 16:1n-7, palmitoleic acid; 18:0, stearic acid; 18:1n-9, oleic acid.

n3-PUFAs may also inhibit their production, by inhibiting conversion of malonyl-CoA to these FAs.[4] Because DNL occurs predominantly in the liver, this process contributes readily to increased intrahepatic fat.[5–7] As such, DNL, rather than direct consumption of these DNL-related FAs, may play a larger role in contributing to higher risk of mortality. This is demonstrated in an 8-week randomized controlled trial among youth diagnosed with nonalcoholic fatty liver disease; lowering free sugars from 11% to 1% of total calories lowered hepatic steatosis by 6.2%.[26] The reduced calories from sugars were mostly replaced with dietary fat, supporting a role of reducing refined carbohydrates and increasing dietary fat to reduce DNL. Thus, possible approaches to reduce circulating levels of these FAs may include reducing intakes of refined starch and sugar, avoiding excess alcohol, and increasing dietary n3-PUFAs.[67] The ability of the liver to handle these incoming substrates can also be modified, such as by changes in lifestyle behaviors to increase physical activity, weight loss, and lean muscle mass (e.g. through resistance training).[68]

In contrast to the other FAs, we identified inverse associations between higher levels of and changes in 18:0 and mortality. Although fewer experimental studies have evaluated this FA, the findings suggest increased inflammation, apoptosis, and endoplasmic reticulum stress.[24,62] On the other hand, in

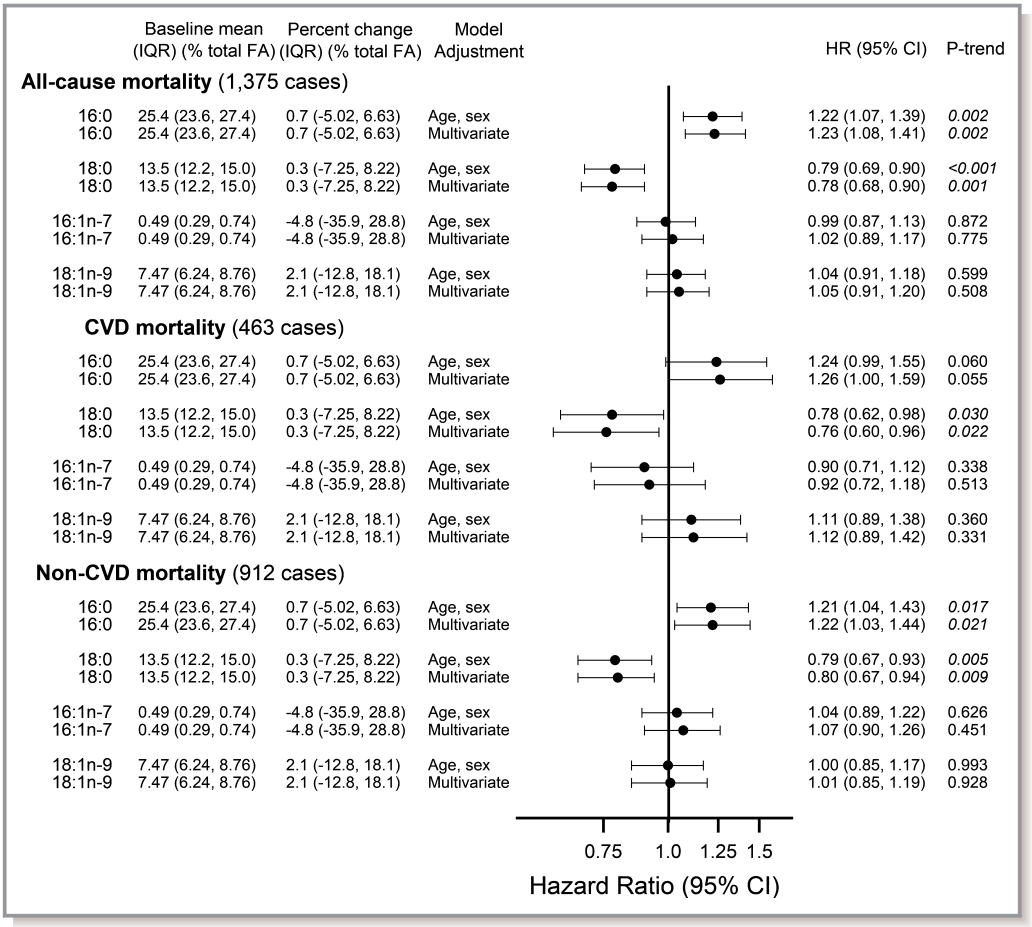

Figure 4. Hazard ratios (and 95% CIs) of all-cause mortality, CVD mortality, and non-CVD mortality events per IQR of percent change for fatty acids from the de novo lipogenesis pathway in the Cardiovascular Health Study after 16 years of maximum follow-up among 1815 older adults. The IQR is estimated to be the difference between the midpoint of the first and fifth quintile. Baseline (IQR), expressed in % total fatty acids, represents the fatty acid levels at study baseline in 1992–1993. Percent change (IQR) is the mean of changes between 1992–1993 and 1998–1999, and 1998–1999 and 2005–2006. Multivariable adjustments additionally include race (white, nonwhite), enrollment site (Bowman Gray, Davis, Hopkins, or Pittsburgh), education (<high school, high school, some college, or college graduate), income (<$11 999, $12 000–$24 999, $25 000–$49 999, or >$50 000/year), body mass index (kg/m^2), physical activity (<500, 500–1000, 1000–1500, or >1500 kcal/week), waist circumference (cm), alcohol intake (0, 0–0.5, 0.5–1, 1–2, 3–7, 8–14, or >14 servings/week), smoking (nonsmokers, former smokers, and current smokers), self-reported health (excellent/very good, good, or fair/poor), and family history of cardiovascular disease (yes, no). CVD, indicates cardiovascular disease; FA, fatty acid; HR, hazard ratio; IQR, interquintile range; 16:0, palmitic acid; 16:1n-7, palmitoleic acid; 18:0, stearic acid; 18:1n-9, oleic acid.

controlled trials of carbohydrate-rich diets, levels of 18:0 were not consistently increased,[33,34] suggesting that it may not be as reliable a biomarker of DNL. Additionally, following adjustment for 16:0 levels, most of the inverse associations noted for 18:0 were attenuated and no longer significant. Thus, the observed protective associations of higher 18:0 levels may be attributed to a correlation with (confounding by) lower 16:0 levels.

18:1n-9 (oleic acid) is the major fatty acid in olive oil, a component of the traditional Mediterranean diet. Although observational studies and randomized controlled trials support the potential cardiometabolic benefit of olive oil,[69] whether oleic acid mediates the beneficial effects is unclear. For example, other nutrients in extra virgin olive oil, such as phenolic compounds, may mediate benefits independent of oleic acid.[70] More importantly, in blood and tissues, levels of oleic acid are influenced by direct production from hepatic DNL in response to carbohydrate-rich diets and increased alcohol intake.[31,33] Consistent with this, estimated dietary intake of oleic acid poorly correlates with circulating oleic acid levels.[35,44] Experimental studies in HepG2 cells demonstrate lipogenic effects of 18:1n-9 independent of stearoyl-CoA desaturase-1[71] as well as apoptotic and steatogenic

properties of 18:1n-9 on hepatocytic cell lines (HepG2, HuH7, and WRL68).[19] Exposure of human hepatocytes to high concentrations of 18:1n-9 also appear to induce steatosis,[22] lending biological support to the positive association between 18:1n-9 and mortality in this study.

The associations between DNL-related FAs and incident total (fatal and nonfatal) CVD were generally more modest than associations with CVD mortality; strongest associations were identified for 16:1n-7 and 18:1n-9 in relation to incident stroke. Assessing subtypes of stroke, these associations remained statistically significant for hemorrhagic stroke, but not ischemic stroke. However, case numbers were low, resulting in wide CIs. Two previous reports identified positive associations between baseline levels of 16:0, 16:1n-7, and 18:1n-9 and ischemic stroke among US adults.[72,73] The present results highlight a need for further study of long-term serial levels of these FAs in relation to stroke subtypes.

We also evaluated, for the first time to our knowledge, the relationship between changes in levels of these FAs and health outcomes. Findings were generally concordant for 16:0 and 18:0, although statistical power overall was more limited in these analyses because of the need to exclude events preceding the time of the second measurement. Our results provide a novel methodological approach to assess risk associated with biomarkers, that appears complementary to usual assessment of baseline or even cumulative levels.

Our findings are internally consistent with past CHS publications which reported a positive association between baseline levels of 16:0 and all-cause mortality (HR, 1.25), and an inverse association between baseline 18:0 and all-cause mortality (0.85),[36] as well as no association between baseline levels of 16:0 and 16:1n-7 with incident CVD.[40] In these studies, only a single baseline FA measure was evaluated, which does not account for potential changes in FA oncentrations over time and may result in attenuation toward the null. The inclusion of repeated FA measurements and cumulative updating in this study reduced measurement error attributable to changes in exposure over time, resulting in more-accurate estimates of long-term exposure with potentially greater relevance to mortality and CVD risk. Consistent with minimized temporal measurements error and bias, we observed stronger associations for 16:0 and 18:0 with mortality (HR for 16:0=1.35; HR for 18:0=0.76), consistent with the expectation that multiple measurements reduced misclassification over time. This likely also explains the differences in null associations using only baseline measures[40] versus repeated measurements of DNL FAs, and incident CHD in the present study.

Few other studies have examined the association between these FAs and mortality. In a Swedish cohort, positive associations were observed between baseline levels of 16:0, 16:1n-7, and 18:1n-9 and risk of total mortality and CVD mortality; 18:0 was not significantly associated with either outcome.[74] Additionally, in a multiethnic US cohort, baseline levels of 18:1n-9 were positively associated with all-cause mortality and incident CVD,[75] consistent with the current study. However, in a cohort of 3591 middle-aged US adults, baseline levels of 16:0 were not significantly associated, whereas 18:0 levels were positively associated, with incident CHD.[43] Our study builds upon and expands these earlier results by including both men and women, focusing on older adults who have the highest risk of mortality, investigating both mortality and incidence of total CVD, evaluating serially measured FA biomarkers over time, and including a longer period of follow-up and larger numbers of events.

The current study has several strengths. This longitudinal cohort of older individuals was followed for nearly a quarter century, and the numbers of events, which were well adjudicated, were large. Additionally, the community-based sample perhaps improves generalizability to older adults. Regular standardized in-person examinations also ensured that covariates were well measured, which may help to minimize confounding. Most importantly, repeated FA biomarker measurements over 13 years allowed the assessment of cumulative effects as well as changes over time in relation to mortality and CVD incidence.

Potential limitations should be considered. Our results have not been validated in a second cohort, and our findings highlight the need for additional studies to evaluate these relationships. Although mortality and incidence were centrally adjudicated, some degree of misclassification is still possible. Some covariates were imputed, which could lead to some degree of imprecision or bias; however, results excluding missing data were similar. The possibility of residual confounding by imprecisely measured or unknown factors cannot be excluded. Although the evaluation of older adults is relevant to risk of mortality and CVD in later life, results may not necessarily be generalizable to younger populations.

Conclusions

Among older adults, higher long-term levels of 16:0, 16:1n-7, and 18:1n-9 and increases in levels of 16:0 were positively, whereas long-term levels and changes in 18:0 were inversely, associated with all-cause mortality. These findings encourage the need for further investigations to understand the independent determinants and effects of these DNL-related FAs, as well as experimental studies to explore novel drug treatments that target DNL.[76]

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Supplemental Material

Data S1.

Supplemental Results

Minor FAs in the DNL pathway

Spearman correlations for serial levels of each minor FAs in the DNL pathway, reflecting reproducibility over time, ranged from 0.37 to 0.63 (Table S12). Pairwise correlations between 14:0, 16:1n-7, and 18:1n-9 were low (r=0.09 to 0.33); 14:0 was negatively correlated to 18:1n-7 (r=-0.00 to -0.20).

Across quintiles, 18:1n-7, but not 16:1n-9 or 14:0, positively associated with all-cause and non-CVD mortality (Figure S8). 16:1n-9 was also positively associated with incident CVD (Figure S9) and incident hemorrhagic stroke (Figure S10), but case numbers were extremely low. Assessed continuously per inter-quintile range (IQR), 18:1n-7 and 16:1n-9 positively associated with all-cause, mortality, while 16:1n-9 was positively associated with CVD, and 18:1n-7 was positively associated with non-CVD mortality (Figure S4). 16:1n-9 was also associated with higher risk of incident CHD (Figure S5). Evaluated either in quintiles or continuously, 14:0 was not associated with incident total CVD nor CVD sub-types (Figures S4 and S5). Restricted cubic splines revealed statistically significant departure from linearity for 14:0 and 16:1n-9 (Figure S11), suggesting possible U-shaped effects.

Non-CVD mortality

We found divergent associations between higher cumulative levels of FAs in the DNL pathway and dementia, pulmonary and other causes of death (Figure S12). For example, per IQR, 18:0 was inversely associated (*P* for trend <0.001), while 16:0 and 18:1n-9 were positively associated with dementia mortality (*P* for trend <0.02 for all). Higher levels of 16:0, 18:1n-9 and 18:1n-7 were associated with a higher risk of cancer, while 16:0, 16:1n-7, 18:1n-9 were positively associated with trauma/fracture mortality, per IQR (*P* for trend <0.05 for all). All FAs were not significantly associated with infection/sepsis mortality.

Most associations between change in all FAs and infection/sepsis and other deaths were not significant (Figure S13). However, divergent associations were identified between these DNL-related FAs and the remaining cause-specific mortality outcomes. For example, 18:0 was inversely associated (*P* for trend = 0.020), while 18:1n-7 was positively associated with cancer mortality (*P* for trend <0.001).

Table S1. Strengthening the reporting of observational studies in epidemiology (STROBE) checklist.

	Item No	Recommendation	Page
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	1
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3-4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4-5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4-5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	4-5
		~~(*b*) For matched studies, give matching criteria and number of exposed and unexposed~~	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Exposure (5) Outcome (7) Confounders/ Potential mediators (6-9)
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4-7
Bias	9	Describe any efforts to address potential sources of bias	4-7
Study size	10	Explain how the study size was arrived at	6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6-9
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7-9
		(*b*) Describe any methods used to examine subgroups and interactions	7-9
		(*c*) Explain how missing data were addressed	8-9
		(*d*) If applicable, explain how loss to follow-up was addressed	7
		(*e*) Describe any sensitivity analyses	9
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	6
		(b) Give reasons for non-participation at each stage	6
		(c) Consider use of a flow diagram	-
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	10, Table 1 & Suppl Table 2-7
		(b) Indicate number of participants with missing data for each variable of interest	8
		(c) Summarise follow-up time (eg, average and total amount)	11
Outcome data	15*	Report numbers of outcome events or summary measures over time	11
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	5, 8-9, Unadjusted: Suppl Table 9, 10 Adjusted: Figure 1, 2
		(*b*) Report category boundaries when continuous variables were categorized	5, 8-9, Unadjusted: Suppl Table 9, 10 Adjusted: Figure 1, 2
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	-
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	12-13
Discussion			
Key results	18	Summarise key results with reference to study objectives	13-14
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	17
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	13-17
Generalisability	21	Discuss the generalisability (external validity) of the study results	17
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	19

Table S2. Baseline characteristics by quintiles of plasma phospholipid palmitic acid (n=3,333) *

	Quintiles of palmitic acid, % total fatty acids				
Variables †	**I (n= 667)**	**II (n= 666)**	**III (n= 666)**	**IV (n= 667)**	**V (n= 667)**
Demographics					
Age, mean (SD), years	74.6 (5.2)	74.6 (5.1)	75.0 (5.4)	75.8 (5.4)	74.7 (5.0)
Female, n (%)	485 (72.7)	395 (59.3)	399 (59.9)	378 (56.7)	418 (62.7)
Race					
White, n (%)	534 (80.1)	560 (84.1)	600 (90.1)	614 (92.1)	614 (92.1)
Non-White, n (%)	133 (1.5)	106 (1.4)	66 (1.2)	53 (1.0)	53 (1.0)
Education, n (%)					
< High school	206 (30.9)	209 (31.4)	156 (23.4)	159 (23.8)	129 (19.3)
High school	207 (31.0)	194 (29.1)	174 (26.1)	173 (25.9)	199 (29.8)
Some college	143 (21.4)	117 (17.6)	188 (28.2)	166 (24.9)	167 (25.0)
College graduate	111 (16.6)	146 (21.9)	148 (22.2)	169 (25.3)	172 (25.8)
Annual income group, n (%)					
<$11,999	210 (31.5)	164 (24.6)	131 (19.7)	145 (21.7)	125 (18.7)
$12,000-$24,999	228 (34.2)	250 (37.5)	239 (35.9)	227 (34.0)	218 (32.7)
$25,000-$49,999	157 (23.5)	165 (24.8)	202 (30.3)	190 (28.5)	214 (32.1)
>$50,000	72 (10.8)	87 (13.1)	94 (14.1)	105 (15.7)	110 (16.5)
Enrollment site, n (%)					
Bowman Gray	237 (35.5)	196 (29.4)	172 (25.8)	132 (19.8)	130 (19.5)
Davis	155 (23.2)	139 (20.9)	170 (25.5)	181 (27.1)	221 (33.1)
Hopkins	156 (23.4)	174 (26.1)	145 (21.8)	157 (23.5)	125 (18.7)
Pittsburgh	119 (17.8)	157 (23.6)	179 (26.9)	197 (29.5)	191 (28.6)
Lifestyle					
Smoking, n (%)					
Current smokers	377 (56.5)	324 (48.6)	324 (48.6)	318 (47.7)	272 (40.8)
Former smokers	223 (33.4)	270 (40.5)	273 (41.0)	291 (43.6)	310 (46.5)
Never smokers	67 (10.0)	72 (10.8)	69 (10.4)	58 (8.7)	85 (12.7)
Physical activity, mean (SD), mcal/week	0.9 (1.2)	1.0 (1.5)	1.1 (1.6)	1.1 (1.5)	1.1 (1.5)
Alcohol, mean (SD), servings/week	0.8 (2.9)	1.8 (4.7)	2.0 (5.7)	3.1 (6.3)	5.3 (9.5)
BMI, mean (SD), kg/m^2	25.9 (4.3)	26.6 (4.7)	27.1 (4.7)	27.0 (5.0)	27.0 (4.5)
Waist circumference, mean (SD), cm	94.6 (12.7)	96.8 (13.2)	97.5 (13.3)	98.4 (13.8)	97.8 (13.0)
Medical History					
Health status (self-report), n (%)					
Excellent/Very good	280 (42.0)	304 (45.6)	304 (45.6)	294 (44.1)	282 (42.3)
Good	239 (35.8)	220 (33.0)	251 (37.7)	257 (38.5)	266 (39.9)
Fair/Poor	148 (22.2)	142 (21.3)	111 (16.7)	116 (17.4)	119 (17.8)
Family history of myocardial infarction or stroke, n (%)	459 (31.2)	459 (31.1)	488 (26.7)	482 (27.7)	480 (28.0)
Incident diabetes, n (%)	46 (6.9)	57 (8.6)	78 (11.7)	75 (11.2)	110 (16.5)
Hypertension medication, n (%)	284 (42.6)	270 (40.5)	249 (37.4)	267 (40.0)	292 (43.8)
Lipid medication, n (%)	34 (5.1)	23 (3.5)	30 (4.5)	22 (3.3)	40 (6.0)
Other Biomarkers					
C-reactive protein, mean (SD), mg/dL	2.7 (4.2)	3.3 (6.5)	3.5 (8.1)	3.2 (4.4)	3.7 (5.3)
High-density lipoprotein, mean (SD), mg/dL	56.0 (13.5)	52.5 (13.3)	52.9 (14.1)	52.4 (13.7)	56.7 (17.0)
Low-density lipoprotein, mean (SD), mg/dL	132.8 (32.5)	128.3 (33.4)	129.8 (31.9)	126.0 (33.5)	119.6 (33.7)
Triglycerides, mean (SD), mg/dL	121.5 (55.9)	130.6 (70.8)	143.1 (85.8)	146.4 (81.5)	172.8 (108.6)
Total ω-3 fatty acid, median (range) ‡, %	4.2 (1.9-12.4)	4.2 (2.0-11.2)	4.2 (2.1-11.5)	4.3 (2.3-11.6)	4.3 (1.9-16.7)
Diet History §					
Energy intake, mean (SD), kcal/d	2,021 (716)	2,028 (669)	2,023 (616)	1,989 (605)	1,968 (565)
Glycemic load, mean (SD), GL units	140 (47)	140 (46)	140 (44)	137 (42)	135 (42)
Fruit intake, mean (SD), servings/d	2.2 (1.0)	2.2 (1.1)	2.3 (1.1)	2.2 (1.1)	2.2 (1.0)
Veg intake, mean (SD), servings/d	3.1 (1.4)	2.9 (1.5)	3.0 (1.5)	2.9 (1.4)	3.0 (1.4)
Processed meat, mean (SD), servings/d	0.4 (0.4)	0.4 (0.4)	0.4 (0.4)	0.4 (0.4)	0.3 (0.3)

* Characteristics reported here are only the 3,333 participants who entered at baseline. The remaining 508 entered at 1998-1999 (year 6), and 28 entered at 2005-06 (year 13), equating to a total of 3,869 participants in the analysis.

† Values reported as mean (SD) for continuous variables, and frequency, (percent) for categorical variables, unless otherwise stated.

‡ Expressed as percentage of total fatty acids.

§ Diet was assessed in 1989-90 and 1995-96 using food frequency questionnaires. Values reported are the mean of both questionnaires.

Table S3. Baseline characteristics by quintiles of plasma phospholipid palmitoleic acid (n=3,333) *

	Quintiles of palmitoleic acid, % total fatty acids				
Variables †	**I (n= 671)**	**II (n= 665)**	**III (n= 665)**	**IV (n= 662)**	**V (n= 670)**
Demographics					
Age, mean (SD), years	74.8 (5.2)	74.9 (5.3)	74.9 (5.2)	75.3 (5.4)	74.8 (5.1)
Female, n (%)	280 (41.7)	369 (55.5)	437 (65.7)	456 (68.9)	533 (79.6)
Race					
White, n (%)	511 (76.2)	561 (84.4)	596 (89.6)	621 (93.8)	633 (94.5)
Non-White, n (%)	160 (1.6)	104 (1.4)	69 (1.2)	41 (0.9)	37 (0.9)
Education, n (%)					
< High school	184 (27.4)	182 (27.4)	184 (27.7)	167 (25.2)	142 (21.2)
High school	175 (26.1)	174 (26.2)	198 (29.8)	196 (29.6)	204 (30.4)
Some college	140 (20.9)	163 (24.5)	154 (23.2)	146 (22.1)	178 (26.6)
College graduate	172 (25.6)	146 (22.0)	129 (19.4)	153 (23.1)	146 (21.8)
Annual income group, n (%)					
<$11,999	161 (24.0)	157 (23.6)	160 (24.1)	146 (22.1)	151 (22.5)
$12,000-$24,999	211 (31.4)	230 (34.6)	230 (34.6)	252 (38.1)	239 (35.7)
$25,000-$49,999	185 (27.6)	182 (27.4)	178 (26.8)	179 (27.0)	204 (30.4)
>$50,000	114 (17.0)	96 (14.4)	97 (14.6)	85 (12.8)	76 (11.3)
Enrollment site, n (%)					
Bowman Gray	205 (30.6)	201 (30.2)	173 (26.0)	139 (21.0)	149 (22.2)
Davis	176 (26.2)	151 (22.7)	164 (24.7)	172 (26.0)	203 (30.3)
Hopkins	111 (16.5)	148 (22.3)	159 (23.9)	180 (27.2)	159 (23.7)
Pittsburgh	179 (26.7)	165 (24.8)	169 (25.4)	171 (25.8)	159 (23.7)
Lifestyle					
Smoking, n (%)					
Current smokers	309 (46.1)	332 (49.9)	331 (49.8)	328 (49.5)	315 (47.0)
Former smokers	301 (44.9)	265 (39.8)	273 (41.1)	269 (40.6)	259 (38.7)
Never smokers	61 (9.1)	68 (10.2)	61 (9.2)	65 (9.8)	96 (14.3)
Physical activity, mean (SD), mcal/week	1.1 (1.5)	1.1 (1.5)	1.1 (1.6)	1.0 (1.4)	0.9 (1.2)
Alcohol, mean (SD), servings/week	2.0 (4.6)	1.7 (5.2)	1.9 (4.8)	2.9 (6.8)	4.4 (9.0)
BMI, mean (SD), kg/m^2	26.3 (4.3)	26.3 (4.3)	26.9 (4.8)	27.2 (4.9)	27.0 (4.9)
Waist circumference, mean (SD), cm	96.7 (11.8)	95.8 (12.9)	97.0 (13.7)	98.1 (13.7)	97.5 (14.1)
Medical History					
Health status (self-report), n (%)					
Excellent/Very good	281 (41.9)	294 (44.2)	295 (44.4)	302 (45.6)	292 (43.6)
Good	239 (35.6)	235 (35.3)	245 (36.8)	250 (37.8)	264 (39.4)
Fair/Poor	151 (22.5)	136 (20.5)	125 (18.8)	110 (16.6)	114 (17.0)
Family history of myocardial infarction or stroke, n (%)	489 (27.1)	469 (29.5)	471 (29.2)	453 (31.6)	486 (27.5)
Incident diabetes, n (%)	61 (9.1)	56 (8.4)	64 (9.6)	85 (12.8)	100 (14.9)
Hypertension medication, n (%)	277 (41.3)	278 (41.8)	273 (41.1)	254 (38.4)	280 (41.8)
Lipid medication, n (%)	24 (3.6)	29 (4.4)	31 (4.7)	23 (3.5)	42 (6.3)
Other Biomarkers					
C-reactive protein, mean (SD), mg/dL	3.1 (6.1)	3.2 (7.2)	3.0 (4.9)	3.6 (6.1)	3.5 (4.9)
High-density lipoprotein, mean (SD), mg/dL	50.9 (13.2)	52.7 (13.4)	54.2 (14.1)	54.0 (14.0)	58.8 (16.3)
Low-density lipoprotein, mean (SD), mg/dL	129.8 (32.4)	130.2 (33.9)	129.1 (34.6)	126.0 (30.6)	121.3 (34.2)
Triglycerides, mean (SD), mg/dL	115.6 (53.5)	131.8 (67.6)	135.9 (77.6)	155.0 (90.1)	176.2 (108.1)
Total ω-3 fatty acid, median (range) ‡, %	4.4 (1.9-12.4)	4.2 (2.2-11.5)	4.2 (1.9-11.6)	4.2 (2.1-16.7)	4.1 (2.1-11.0)
Diet History §					
Energy intake, mean (SD), kcal/d	2,072 (784)	2,064 (607)	1,987 (610)	1,982 (591)	1,924 (554)
Glycemic load, mean (SD), GL units	141 (51)	142 (42)	138 (44)	138 (42)	134 (41)
Fruit intake, mean (SD), servings/d	2.1 (1.1)	2.2 (1.0)	2.3 (1.0)	2.3 (1.1)	2.3 (1.1)
Veg intake, mean (SD), servings/d	3.0 (1.5)	3.0 (1.4)	3.0 (1.4)	3.0 (1.5)	3.0 (1.4)
Processed meat, mean (SD), servings/d	0.4 (0.4)	0.4 (0.4)	0.4 (0.4)	0.3 (0.3)	0.3 (0.3)

* Characteristics reported here are only the 3,333 participants who entered at baseline. The remaining 508 entered at 1998-1999 (year 6), and 28 entered at 2005-06 (year 13), equating to a total of 3,869 participants in the analysis.

† Values reported as mean (SD) for continuous variables, and frequency, (percent) for categorical variables, unless otherwise stated.

‡ Expressed as percentage of total fatty acids.

§ Diet was assessed in 1989-90 and 1995-96 using food frequency questionnaires. Values reported are the mean of both questionnaires.

Table S4. Baseline characteristics by quintiles of plasma phospholipid stearic acid (n=3,333) *

	Quintiles of stearic acid, % total fatty acids				
Variables †	**I (n= 668)**	**II (n= 666)**	**III (n= 665)**	**IV (n= 667)**	**V (n= 667)**
Demographics					
Age, mean (SD), years	75.9 (5.7)	75.4 (5.3)	75.1 (5.2)	74.7 (5.1)	73.6 (4.4)
Female, n (%)	361 (54.0)	384 (57.7)	369 (55.5)	452 (67.8)	509 (76.3)
Race					
White, n (%)	618 (92.5)	592 (88.9)	581 (87.4)	579 (86.8)	552 (82.8)
Non-White, n (%)	50 (1.0)	74 (1.2)	84 (1.3)	88 (1.3)	115 (1.5)
Education, n (%)					
< High school	144 (21.6)	156 (23.4)	155 (23.3)	198 (29.7)	206 (30.9)
High school	190 (28.4)	200 (30.0)	188 (28.3)	179 (26.8)	190 (28.5)
Some college	149 (22.3)	149 (22.4)	168 (25.3)	160 (24.0)	155 (23.2)
College graduate	185 (27.7)	161 (24.2)	154 (23.2)	130 (19.5)	116 (17.4)
Annual income group, n (%)					
<$11,999	120 (18.0)	146 (21.9)	150 (22.6)	172 (25.8)	187 (28.0)
$12,000-$24,999	228 (34.1)	247 (37.1)	224 (33.7)	227 (34.0)	236 (35.4)
$25,000-$49,999	219 (32.8)	182 (27.3)	196 (29.5)	172 (25.8)	159 (23.8)
>$50,000	101 (15.1)	91 (13.7)	95 (14.3)	96 (14.4)	85 (12.7)
Enrollment site, n (%)					
Bowman Gray	169 (25.3)	157 (23.6)	177 (26.6)	153 (22.9)	211 (31.6)
Davis	195 (29.2)	178 (26.7)	161 (24.2)	178 (26.7)	154 (23.1)
Hopkins	130 (19.5)	133 (20.0)	162 (24.4)	170 (25.5)	162 (24.3)
Pittsburgh	174 (26.0)	198 (29.7)	165 (24.8)	166 (24.9)	140 (21.0)
Lifestyle					
Smoking, n (%)					
Current smokers	301 (45.1)	311 (46.7)	311 (46.8)	342 (51.3)	350 (52.5)
Former smokers	303 (45.4)	275 (41.3)	276 (41.5)	273 (40.9)	240 (36.0)
Never smokers	64 (9.6)	80 (12.0)	78 (11.7)	52 (7.8)	77 (11.5)
Physical activity, mean (SD), mcal/week	1.2 (1.6)	1.1 (1.5)	1.1 (1.5)	1.0 (1.4)	0.9 (1.3)
Alcohol, mean (SD), servings/week	3.3 (6.3)	2.8 (6.9)	2.4 (6.1)	2.6 (7.0)	1.9 (5.3)
BMI, mean (SD), kg/m^2	25.3 (4.2)	26.1 (4.5)	26.8 (4.8)	27.6 (4.8)	27.9 (4.6)
Waist circumference, mean (SD), cm	93.7 (12.5)	95.4 (12.9)	97.0 (13.3)	99.2 (13.6)	99.8 (13.1)
Medical History					
Health status (self-report), n (%)					
Excellent/Very good	292 (43.7)	299 (44.9)	303 (45.6)	287 (43.0)	283 (42.4)
Good	248 (37.1)	237 (35.6)	246 (37.0)	244 (36.6)	258 (38.7)
Fair/Poor	128 (19.2)	130 (19.5)	116 (17.4)	136 (20.4)	126 (18.9)
Family history of myocardial infarction or stroke, n (%)	491 (26.5)	484 (27.3)	478 (28.1)	469 (29.7)	446 (33.1)
Incident diabetes, n (%)	75 (11.2)	63 (9.5)	77 (11.6)	71 (10.6)	80 (12.0)
Hypertension medication, n (%)	245 (36.7)	259 (38.9)	263 (39.5)	286 (42.9)	309 (46.3)
Lipid medication, n (%)	21 (3.1)	30 (4.5)	21 (3.2)	37 (5.5)	40 (6.0)
Other Biomarkers					
C-reactive protein, mean (SD), mg/dL	3.8 (8.1)	2.7 (5.8)	3.2 (5.4)	3.1 (4.2)	3.5 (5.3)
High-density lipoprotein, mean (SD), mg/dL	59.5 (17.1)	54.8 (14.9)	53.1 (13.5)	52.4 (13.1)	50.7 (11.6)
Low-density lipoprotein, mean (SD), mg/dL	115.7 (31.8)	125.8 (32.2)	128.4 (32.4)	131.1 (34.3)	135.6 (32.4)
Triglycerides, mean (SD), mg/dL	130.8 (89.6)	126.4 (77.8)	136.8 (78.2)	149.5 (80.4)	170.9 (86.4)
Total ω-3 fatty acid, median (range) ‡, %	4.1 (1.9-16.7)	4.1 (2.0-11.1)	4.2 (2.4-11.6)	4.3 (2.4-12.4)	4.4 (2.4-11.6)
Diet History §					
Energy intake, mean (SD), kcal/d	1,994 (614)	1,991 (585)	2,041 (657)	1,994 (635)	2,010 (688)
Glycemic load, mean (SD), GL units	138 (43)	138 (42)	140 (45)	137 (44)	140 (46)
Fruit intake, mean (SD), servings/d	2.2 (1.1)	2.3 (1.1)	2.2 (1.1)	2.2 (1.0)	2.2 (1.0)
Veg intake, mean (SD), servings/d	2.9 (1.4)	2.9 (1.4)	3.0 (1.5)	2.9 (1.5)	3.1 (1.5)
Processed meat, mean (SD), servings/d	0.3 (0.4)	0.4 (0.4)	0.4 (0.4)	0.4 (0.4)	0.4 (0.4)

* Characteristics reported here are only the 3,333 participants who entered at baseline. The remaining 508 entered at 1998-1999 (year 6), and 28 entered at 2005-06 (year 13), equating to a total of 3,869 participants in the analysis.

† Values reported as mean (SD) for continuous variables, and frequency, (percent) for categorical variables, unless otherwise stated.

‡ Expressed as percentage of total fatty acids.

§ Diet was assessed in 1989-90 and 1995-96 using food frequency questionnaires. Values reported are the mean of both questionnaires.

Table S5. Baseline characteristics by quintiles of plasma phospholipid oleic acid (n=3,333) *

	Quintiles of oleic acid, % total fatty acids				
Variables †	**I (n= 666)**	**II (n= 667)**	**III (n= 666)**	**IV (n= 668)**	**V (n= 666)**
Demographics					
Age, mean (SD), years	74.1 (4.7)	74.7 (5.0)	75.2 (5.3)	75.2 (5.5)	75.6 (5.5)
Female, n (%)	428 (64.3)	428 (64.2)	421 (63.2)	414 (62.0)	384 (57.7)
Race					
White, n (%)	527 (79.1)	588 (88.2)	596 (89.5)	608 (91.0)	603 (90.5)
Non-White, n (%)	139 (1.6)	79 (1.3)	70 (1.2)	60 (1.1)	63 (1.1)
Education, n (%)					
< High school	159 (23.9)	166 (24.9)	194 (29.1)	174 (26.0)	166 (24.9)
High school	206 (30.9)	193 (28.9)	193 (29.0)	171 (25.6)	184 (27.6)
Some college	149 (22.4)	165 (24.7)	131 (19.7)	177 (26.5)	159 (23.9)
College graduate	152 (22.8)	143 (21.4)	148 (22.2)	146 (21.9)	157 (23.6)
Annual income group, n (%)					
<$11,999	174 (26.1)	151 (22.6)	153 (23.0)	139 (20.8)	158 (23.7)
$12,000-$24,999	204 (30.6)	226 (33.9)	248 (37.2)	243 (36.4)	241 (36.2)
$25,000-$49,999	174 (26.1)	187 (28.0)	184 (27.6)	197 (29.5)	186 (27.9)
>$50,000	114 (17.1)	103 (15.4)	81 (12.2)	89 (13.3)	81 (12.2)
Enrollment site, n (%)					
Bowman Gray	185 (27.8)	191 (28.6)	185 (27.8)	157 (23.5)	149 (22.4)
Davis	142 (21.3)	167 (25.0)	173 (26.0)	195 (29.2)	189 (28.4)
Hopkins	138 (20.7)	147 (22.0)	153 (23.0)	150 (22.5)	169 (25.4)
Pittsburgh	201 (30.2)	162 (24.3)	155 (23.3)	166 (24.9)	159 (23.9)
Lifestyle					
Smoking, n (%)					
Current smokers	323 (48.5)	359 (53.8)	317 (47.6)	316 (47.3)	300 (45.0)
Former smokers	291 (43.7)	255 (38.2)	284 (42.6)	281 (42.1)	256 (38.4)
Never smokers	52 (7.8)	53 (7.9)	65 (9.8)	71 (10.6)	110 (16.5)
Physical activity, mean (SD), mcal/week	1.0 (1.4)	1.1 (1.5)	1.1 (1.4)	1.1 (1.4)	1.0 (1.5)
Alcohol, mean (SD), servings/week	1.5 (3.7)	2.1 (6.2)	2.1 (5.8)	2.8 (5.8)	4.5 (8.9)
BMI, mean (SD), kg/m^2	27.5 (4.8)	26.9 (4.6)	27.0 (4.6)	26.5 (4.6)	25.8 (4.6)
Waist circumference, mean (SD), cm	98.4 (13.1)	97.0 (12.8)	97.9 (13.1)	96.6 (13.6)	95.1 (13.4)
Medical History					
Health status (self-report), n (%)					
Excellent/Very good	281 (42.2)	315 (47.2)	288 (43.2)	298 (44.6)	282 (42.3)
Good	254 (38.1)	233 (34.9)	250 (37.5)	238 (35.6)	258 (38.7)
Fair/Poor	131 (19.7)	119 (17.8)	128 (19.2)	132 (19.8)	126 (18.9)
Family history of myocardial infarction or stroke, n (%)	472 (29.1)	468 (29.8)	462 (30.6)	472 (29.3)	494 (25.8)
Incident diabetes, n (%)	80 (12.0)	65 (9.7)	77 (11.6)	70 (10.5)	74 (11.1)
Hypertension medication, n (%)	305 (45.8)	280 (42.0)	267 (40.1)	264 (39.5)	246 (36.9)
Lipid medication, n (%)	33 (5.0)	21 (3.1)	37 (5.6)	30 (4.5)	28 (4.2)
Other Biomarkers					
C-reactive protein, mean (SD), mg/dL	3.3 (6.0)	3.1 (4.0)	3.6 (7.5)	3.1 (5.4)	3.3 (6.0)
High-density lipoprotein, mean (SD), mg/dL	53.0 (13.8)	53.0 (13.3)	53.3 (13.9)	55.1 (14.8)	56.1 (16.2)
Low-density lipoprotein, mean (SD), mg/dL	133.9 (34.2)	132.9 (31.4)	125.8 (31.8)	122.7 (33.4)	121.2 (33.7)
Triglycerides, mean (SD), mg/dL	124.9 (60.4)	138.4 (79.5)	147.4 (77.5)	145.1 (79.4)	158.6 (112.1)
Total ω-3 fatty acid, median (range) ‡, %	4.6 (2.4-16.7)	4.4 (2.3-10.6)	4.2 (2.0-11.6)	4.0 (2.3-11.0)	3.9 (1.9-8.6)
Diet History §					
Energy intake, mean (SD), kcal/d	2,004 (714)	1,986 (595)	2,000 (618)	2,028 (627)	2,010 (624)
Glycemic load, mean (SD), GL units	139 (47)	138 (42)	138 (42)	140 (46)	137 (43)
Fruit intake, mean (SD), servings/d	2.3 (1.1)	2.3 (1.1)	2.2 (1.0)	2.2 (1.1)	2.2 (1.1)
Veg intake, mean (SD), servings/d	3.1 (1.5)	3.1 (1.4)	2.9 (1.4)	2.9 (1.5)	2.9 (1.5)
Processed meat, mean (SD), servings/d	0.4 (0.4)	0.4 (0.3)	0.4 (0.3)	0.4 (0.4)	0.4 (0.4)

* Characteristics reported here are only the 3,333 participants who entered at baseline. The remaining 508 entered at 1998-1999 (year 6), and 28 entered at 2005-06 (year 13), equating to a total of 3,869 participants in the analysis.

† Values reported as mean (SD) for continuous variables, and frequency, (percent) for categorical variables, unless otherwise stated.

‡ Expressed as percentage of total fatty acids.

§ Diet was assessed in 1989-90 and 1995-96 using food frequency questionnaires. Values reported are the mean of both questionnaires.

Table S6. Participant characteristics at study baseline in 1992-93 for participants who were deceased before study baseline, with no fatty acid measurements for all time periods, and those included in the analysis at baseline in the Cardiovascular Health Study.

Variables	Deceased before study baseline (n=592)	No fatty acid measurements (n=737)	Included in analysis at baseline (n=3,869)
Age, mean (SD), years	78 (7)	77 (7)	75 (5)
Female, n (%)	273 (46%)	426 (58%)	2,402 (62%)
Ethnicity			
White, n (%)	548 (93%)	528 (72%)	3,278 (85%)
Non-White, n (%)	44 (7%)	209 (28%)	591 (15%)
Education, n (%)			
< High school	249 (42%)	272 (37%)	988 (26%)
High school	150 (25%)	188 (26%)	1,112 (29%)
Some college	109 (18%)	172 (23%)	905 (23%)
College graduate	84 (14%)	105 (14%)	864 (22%)
Annual income group, n (%)			
<$12,000	190 (32%)	266 (36%)	889 (23%)
$12,000-$24,999	230 (39%)	272 (37%)	1,360 (35%)
$25,000-$49,999	128 (22%)	140 (19%)	1,088 (28%)
>$50,000	44 (7%)	59 (8%)	532 (14%)
Enrolment site, n (%)			
Bowman Gray, NC	223 (38%)	177 (24%)	1,013 (26%)
Davis, CA	80 (14%)	224 (30%)	1,051 (27%)
Hopkins, MD	160 (27%)	146 (20%)	826 (21%)
Pittsburgh, PA	129 (22%)	190 (26%)	979 (25%)
Smoking, n (%)			
Never smokers	236 (40%)	343 (47%)	1,882 (49%)
Former smokers	255 (43%)	278 (38%)	1,578 (41%)
Current smokers	101 (17%)	116 (16%)	409 (11%)
Physical activity, mean (SD), kcal/week	886 (1284)	889 (1381)	1160 (1373)
Alcohol, mean (SD), servings/week	1.9 (5.0)	2.9 (24)	2.6 (6.4)
BMI, mean (SD), kg/m^2	26.5 (2.1)	27.1 (4.6)	26.8 (4.7)
Waist circumference, mean (SD), cm	98.0 (5.5)	98.5 (12)	97.2 (13)
Health status (self-report), n (%)			
Excellent/Very good	136 (23%)	215 (29%)	1,697 (44%)
Good	205 (35%)	260 (35%)	1,450 (37%)
Fair/Poor	251 (42%)	262 (36%)	722 (19%)
Family history of myocardial infarction or stroke, n (%)	188 (32%)	213 (29%)	1,127 (29%)

Table S7. Participant characteristics at study baseline in 1992-93 for participants by the occurrence of all-cause mortality in the Cardiovascular Health Study.

Variables	All-cause mortality cases (n=3,227)	All-cause mortality non-cases (n=642)
Age, mean (SD), years	75 (5)	71 (3)
Female, n (%)	1,943 (60%)	459 (72%)
Ethnicity		
White, n (%)	2,761 (86%)	517 (81%)
Non-White, n (%)	466 (14%)	125 (19%)
Education, n (%)		
< High school	867 (27%)	121 (19%)
High school	918 (28%)	194 (30%)
Some college	741 (23%)	164 (26%)
College graduate	701 (22%)	163 (25%)
Annual income group, n (%)		
<$12,000	768 (24%)	121 (19%)
$12,000-$24,999	1,187 (37%)	173 (27%)
$25,000-$49,999	863 (27%)	225 (35%)
>$50,000	409 (13%)	123 (29%)
Enrolment site, n (%)		
Bowman Gray, NC	832 (26%)	181 (28%)
Davis, CA	856 (27%)	195 (30%)
Hopkins, MD	719 (22%)	107 (17%)
Pittsburgh, PA	820 (25%)	159 (25%)
Smoking, n (%)		
Never smokers	1,505 (47%)	377 (59%)
Former smokers	1,358 (42%)	220 (34%)
Current smokers	364 (11%)	45 (7%)
Physical activity, mean (SD), kcal/week	1140 (1366)	1256 (1405)
Alcohol, mean (SD), servings/week	2.7 (6.6)	2.4 (5.3)
BMI, mean (SD), kg/m^2	26.8 (4.7)	27.1 (4.5)
Waist circumference, mean (SD), cm	97.4 (13)	96.2 (13)
Health status (self-report), n (%)		
Excellent/Very good	1,360 (42%)	337 (52%)
Good	1,217 (38%)	233 (36%)
Fair/Poor	650 (20%)	72 (11%)
Family history of myocardial infarction or stroke, n (%)	944 (29%)	183 (29%)

Table S8. Unadjusted spearman pairwise correlation coefficients for major plasma phospholipid fatty acids in the de novo lipogenesis pathway among 3,869 adults.

		Palmitic acid (16:0)			Palmitoleic acid (16:1n-7)			Stearic acid (18:0)			Oleic acid (18:1n-9)		
		Year 5	Year 11	Year 18	Year 5	Year 11	Year 18	Year 5	Year 11	Year 18	Year 5	Year 11	Year 18
16:0	Year 5	1.00											
	Year 11	0.62	1.00										
	Year 18	0.54	0.52	1.00									
16:1n-7	Year 5	0.57	0.39	0.24	1.00								
	Year 11	0.35	0.54	0.26	0.67	1.00							
	Year 18	0.32	0.34	0.45	0.54	0.63	1.00						
18:0	Year 5	-0.45	-0.33	-0.33	-0.23	-0.21	-0.18	1.00					
	Year 11	-0.35	-0.50	-0.37	-0.21	-0.27	-0.20	0.64	1.00				
	Year 18	-0.32	-0.28	-0.58	-0.10*	-0.08	-0.23	0.53	0.53	1.00			
18:1n-9	Year 5	0.32	0.20	0.15	0.57	0.33	0.30	-0.28	-0.25	-0.19	1.00		
	Year 11	0.21	0.26	0.23	0.31	0.52	0.34	-0.20	-0.28	-0.24	0.51	1.00	
	Year 18	0.23	0.20	0.33	0.23	0.28	0.56	-0.18	-0.21	-0.32	0.40	0.44	1.00

* All correlations were significant (P<0.05), except for values with an asterisk

Table S9. Adjusted partial correlations (age and sex) between major fatty acids in the de novo lipogenesis pathway and cardiovascular disease risk factors at baseline in 1992-1993.

Year 5 (n=3,333)	**Palmitic acid (16:0)**	**Palmitoleic acid (16:1n-7)**	**Stearic acid (18:0)**	**Oleic acid (18:1n-9)**
LDL-C	-0.13	-0.16	0.17	-0.15
HDL-C	0.07	0.14	-0.29	0.13
Triglycerides	0.21	0.23	0.16	0.12
SBP	0.01*	0.03*	0.01*	0.02*
Fasting glucose	0.13	-0.002*	-0.03*	-0.02*

* All correlations were significant (P<0.05), except for highlighted values

LDL-C = Low density lipoprotein cholesterol; HDL-C = High density lipoprotein cholesterol; SBP = systolic blood pressure

Table S10. Unadjusted analyses for major fatty acids from the de novo lipogenesis pathway and the risk of all-cause mortality, cardiovascular mortality and non-cardiovascular mortality in the Cardiovascular Health Study after 22 years of maximum follow-up among 3,869 older adults.

	Quintiles of fatty acid					*P*
All-cause mortality	I	II	III	IV	V	trend*
Palmitic acid (16:0)						
Events/Total (per 1,000 person-years)	530/669 (59)	612/748 (65)	699/836 (70)	719/850 (74)	667/766 (75)	
Median % of total fatty acids	23.54	24.56	25.25	26.06	27.43	
Unadjusted, HR (95% CI)	1.00 (Ref)	1.09 (0.97-1.22)	1.17 (1.05-1.31)	1.24 (1.11-1.39)	1.31 (1.17-1.47)	<0.001
Stearic acid (18:0)						
Events/Total (per 1,000 person-years)	613/691 (78)	677/790 (77)	609/725 (68)	716/883 (66)	612/780 (58)	
Median % of total fatty acids	12.13	12.90	13.41	13.99	14.81	
Unadjusted, HR (95% CI)	1.00 (Ref)	0.93 (0.84-1.04)	0.82 (0.73-0.92)	0.74 (0.67-0.83)	0.65 (0.58-0.73)	<0.001
Palmitoleic acid (16:1n-7)						
Events/Total (per 1,000 person-years)	753/942 (69)	697/838 (70)	617/736 (67)	592/690 (70)	568/663 (68)	
Median % of total fatty acids	0.28	0.37	0.45	0.55	0.73	
Unadjusted, HR (95% CI)	1.00 (Ref)	1.05 (0.95-1.16)	1.05 (0.94-1.16)	1.13 (1.01-1.25)	1.11 (1.00-1.24)	0.028
Oleic acid (18:1n-9)						
Events/Total (per 1,000 person-years)	541/692 (54)	615/756 (60)	702/845 (73)	719/837 (80)	650/739 (81)	
Median % of total fatty acids	6.36	6.95	7.45	8.01	8.91	
Unadjusted, HR (95% CI)	1.00 (Ref)	1.12 (1.00-1.26)	1.36 (1.21-1.52)	1.52 (1.36-1.70)	1.63 (1.45-1.83)	<0.001
Cardiovascular mortality						
Palmitic acid (16:0)						
Events/Total (per 1,000 person-years)	196/669 (22)	197/748 (21)	242/836 (24)	248/850 (26)	248/766 (28)	
Median % of total fatty acids	23.54	24.56	25.25	26.06	27.43	
Unadjusted, HR (95% CI)	1.00 (Ref)	0.95 (0.78-1.16)	1.10 (0.91-1.33)	1.16 (0.97-1.40)	1.31 (1.09-1.58)	<0.001
Stearic acid (18:0)						
Events/Total (per 1,000 person-years)	205/691 (26)	249/790 (28)	210/725 (24)	252/883 (23)	215/780 (20)	
Median % of total fatty acids	12.13	12.90	13.41	13.99	14.81	
Unadjusted, HR (95% CI)	1.00 (Ref)	1.03 (0.86-1.24)	0.85 (0.70-1.03)	0.80 (0.66-0.96)	0.69 (0.57-0.84)	<0.001
Palmitoleic acid (16:1n-7)						
Events/Total (per 1,000 person-years)	255/942 (23)	243/838 (24)	222/736 (24)	221/690 (26)	190/663 (23)	
Median % of total fatty acids	0.28	0.37	0.45	0.55	0.73	
Unadjusted, HR (95% CI)	1.00 (Ref)	1.08 (0.90-1.28)	1.10 (0.92-1.32)	1.23 (1.02-1.47)	1.08 (0.90-1.31)	0.256
Oleic acid (18:1n-9)						
Events/Total (per 1,000 person-years)	189/692 (19)	199/756 (19)	260/845 (27)	262/837 (29)	221/739 (28)	
Median % of total fatty acids	6.36	6.95	7.45	8.01	8.91	
Unadjusted, HR (95% CI)	1.00 (Ref)	1.04 (0.85-1.27)	1.44 (1.19-1.73)	1.59 (1.32-1.91)	1.57 (1.29-1.90)	<0.001
Non-cardiovascular mortality						
Palmitic acid (16:0)						
Events/Total (per 1,000 person-years)	334/669 (37)	415/748 (44)	457/836 (46)	471/850 (49)	419/766 (47)	
Median % of total fatty acids	23.54	24.56	25.25	26.06	27.43	
Unadjusted, HR (95% CI)	1.00 (Ref)	1.17 (1.01-1.35)	1.21 (1.05-1.40)	1.29 (1.12-1.49)	1.31 (1.13-1.51)	<0.001
Stearic acid (18:0)						
Events/Total (per 1,000 person-years)	408/691 (52)	428/790 (48)	399/725 (45)	464/883 (43)	397/780 (37)	
Median % of total fatty acids	12.13	12.90	13.41	13.99	14.81	
Unadjusted, HR (95% CI)	1.00 (Ref)	0.88 (0.77-1.01)	0.80 (0.70-0.92)	0.72 (0.63-0.82)	0.63 (0.55-0.72)	<0.001
Palmitoleic acid (16:1n-7)						
Events/Total (per 1,000 person-years)	498/942 (45)	454/838 (45)	395/736 (43)	371/690 (44)	378/663 (45)	
Median % of total fatty acids	0.28	0.37	0.45	0.55	0.73	
Unadjusted, HR (95% CI)	1.00 (Ref)	1.04 (0.91-1.18)	1.02 (0.89-1.16)	1.07 (0.94-1.23)	1.13 (0.99-1.29)	0.059
Oleic acid (18:1n-9)						
Events/Total (per 1,000 person-years)	352/692 (35)	416/756 (41)	442/845 (46)	457/837 (51)	429/739 (53)	
Median % of total fatty acids	6.36	6.95	7.45	8.01	8.91	
Unadjusted, HR (95% CI)	1.00 (Ref)	1.17 (1.01-1.34)	1.31 (1.14-1.51)	1.49 (1.30-1.71)	1.66 (1.44-1.91)	<0.001

* P trend was calculated by assessing quintiles as continuous variables after assigning participants the median value in each quintile.
CI = confidence interval; HR = hazard ratio

Table S11. Unadjusted analyses for major fatty acids from the de novo lipogenesis pathway and the risk of fatal and non-fatal cardiovascular disease, coronary heart disease (CHD) and stroke in the Cardiovascular Health Study after 22 years of maximum follow-up among 3,869 older adults.

	Quintiles of fatty acid					*P*
Fatal and non-fatal CVD	I	II	III	IV	V	trend*
Palmitic acid (16:0)						
Events/Total (per 1,000 person-years)	309/670 (39)	325/753 (39)	384/838 (44)	371/839 (44)	364/769 (46)	
Median % of total fatty acids	23.53	24.56	25.26	26.07	27.46	
Unadjusted, HR (95% CI)	1.00 (Ref)	0.99 (0.85-1.16)	1.11 (0.96-1.30)	1.11 (0.95-1.29)	1.20 (1.03-1.39)	0.007
Stearic acid (18:0)						
Events/Total (per 1,000 person-years)	323/707 (46)	373/778 (48)	332/748 (42)	374/860 (40)	351/776 (38)	
Median % of total fatty acids	12.12	12.89	13.40	13.99	14.81	
Unadjusted, HR (95% CI)	1.00 (Ref)	1.02 (0.88-1.18)	0.87 (0.75-1.02)	0.80 (0.69-0.93)	0.76 (0.66-0.89)	<0.001
Palmitoleic acid (16:1n-7)						
Events/Total (per 1,000 person-years)	406/929 (42)	381/850 (43)	341/730 (42)	317/683 (42)	308/677 (41)	
Median % of total fatty acids	0.28	0.37	0.45	0.55	0.73	
Unadjusted, HR (95% CI)	1.00 (Ref)	1.04 (0.90-1.19)	1.02 (0.88-1.17)	1.05 (0.91-1.22)	1.03 (0.89-1.20)	0.713
Oleic acid (18:1n-9)						
Events/Total (per 1,000 person-years)	333/711 (37)	340/768 (38)	365/828 (42)	381/824 (48)	334/738 (48)	
Median % of total fatty acids	6.36	6.95	7.45	8.01	8.91	
Unadjusted, HR (95% CI)	1.00 (Ref)	1.01 (0.86-1.17)	1.14 (0.98-1.32)	1.31 (1.13-1.52)	1.33 (1.14-1.55)	<0.001
Fatal and non-fatal CHD						
Palmitic acid (16:0)						
Events/Total (per 1,000 person-years)	212/667 (25)	236/752 (27)	276/843 (30)	247/842 (28)	233/765 (28)	
Median % of total fatty acids	23.53	24.56	25.26	26.07	27.44	
Unadjusted, HR (95% CI)	1.00 (Ref)	1.06 (0.88-1.28)	1.18 (0.98-1.41)	1.08 (0.89-1.29)	1.12 (0.93-1.35)	0.280
Stearic acid (18:0)						
Events/Total (per 1,000 person-years)	208/701 (29)	258/786 (32)	216/735 (26)	269/865 (27)	253/782 (26)	
Median % of total fatty acids	12.13	12.89	13.40	13.99	14.81	
Unadjusted, HR (95% CI)	1.00 (Ref)	1.09 (0.91-1.31)	0.88 (0.73-1.07)	0.89 (0.74-1.07)	0.85 (0.71-1.02)	0.013
Palmitoleic acid (16:1n-7)						
Events/Total (per 1,000 person-years)	300/933 (30)	259/840 (28)	247/739 (29)	212/684 (27)	186/673 (24)	
Median % of total fatty acids	0.28	0.37	0.45	0.55	0.73	
Unadjusted, HR (95% CI)	1.00 (Ref)	0.96 (0.81-1.13)	1.00 (0.84-1.18)	0.95 (0.79-1.13)	0.84 (0.70-1.01)	0.068
Oleic acid (18:1n-9)						
Events/Total (per 1,000 person-years)	230/702 (25)	236/765 (25)	258/834 (29)	257/827 (31)	223/741 (30)	
Median % of total fatty acids	6.36	6.95	7.45	8.01	8.91	
Unadjusted, HR (95% CI)	1.00 (Ref)	1.00 (0.84-1.21)	1.16 (0.97-1.38)	1.27 (1.06-1.52)	1.27 (1.06-1.53)	0.001
Fatal and non-fatal stroke						
Palmitic acid (16:0)						
Events/Total (per 1,000 person-years)	125/670 (15)	121/750 (13)	139/831 (15)	155/848 (17)	143/770 (17)	
Median % of total fatty acids	23.54	24.56	25.25	26.06	27.45	
Unadjusted, HR (95% CI)	1.00 (Ref)	0.90 (0.70-1.15)	0.98 (0.77-1.24)	1.12 (0.89-1.42)	1.13 (0.89-1.44)	0.085
Stearic acid (18:0)						
Events/Total (per 1,000 person-years)	133/702 (18)	137/779 (16)	136/738 (16)	138/878 (14)	139/772 (14)	
Median % of total fatty acids	12.13	12.90	13.41	13.99	14.82	
Unadjusted, HR (95% CI)	1.00 (Ref)	0.90 (0.71-1.14)	0.88 (0.69-1.12)	0.73 (0.57-0.92)	0.75 (0.59-0.95)	0.005
Palmitoleic acid (16:1n-7)						
Events/Total (per 1,000 person-years)	145/937 (14)	150/848 (16)	126/725 (14)	124/690 (16)	138/669 (17)	
Median % of total fatty acids	0.28	0.37	0.45	0.55	0.73	
Unadjusted, HR (95% CI)	1.00 (Ref)	1.14 (0.91-1.43)	1.05 (0.83-1.34)	1.15 (0.91-1.47)	1.29 (1.02-1.63)	0.042
Oleic acid (18:1n-9)						
Events/Total (per 1,000 person-years)	124/702 (13)	133/759 (14)	142/839 (15)	149/831 (18)	135/738 (18)	
Median % of total fatty acids	6.36	6.94	7.45	8.01	8.91	
Unadjusted, HR (95% CI)	1.00 (Ref)	1.06 (0.83-1.36)	1.19 (0.94-1.52)	1.36 (1.08-1.73)	1.40 (1.10-1.79)	0.001

* P trend was calculated by assessing quintiles as continuous variables after assigning participants the median value in each quintile.
CHD = coronary heart disease; CI = confidence interval; CVD = cardiovascular disease; HR = hazard ratio

Table S12. Unadjusted spearman pairwise correlation coefficients for minor plasma phospholipid fatty acids in the de novo lipogenesis pathway among 3,869 adults.

		Myristic acid (14:0)			7-hexadecenoic acid (16:1n-9)			Vaccenic acid (18:1n-7)		
		Year 5	Year 11	Year 18	Year 5	Year 11	Year 18	Year 5	Year 11	Year 18
14:0	Year 5	1.00								
	Year 11	0.47	1.00							
	Year 18	0.37	0.50	1.00						
16:1n-9	Year 5	0.20	0.13	0.11	1.00					
	Year 11	0.09	0.15	0.12	0.49	1.00				
	Year 18	0.10	0.15	0.18	0.38	0.48	1.00			
18:1n-7	Year 5	-0.20	-0.03*	0.00*	0.33	0.27	0.22	1.00		
	Year 11	-0.04*	-0.18	-0.05*	0.28	0.42	0.31	0.63	1.00	
	Year 18	-0.03*	-0.03*	-0.23	0.21	0.30	0.43	0.49	0.59	1.00

* All correlations were significant (P<0.05), except for values with an asterisk

Table S13. Major fatty acids from the de novo lipogenesis pathway and the risk of all-cause mortality in the Cardiovascular Health Study: analysis of potential interaction by age, sex, body mass index, waist circumference and self-reported health, with respective stratified analyses with Bonferroni correction (significance <0.001)*

	Palmitic acid (16:0)		Stearic acid (18:0)		Palmitoleic acid (16:1n-7)		Oleic aicd (18:1n-9)	
	HR (95% CI)	P-value †	HR (95% CI)	P-value †	HR (95% CI)	P-value †	HR (95% CI)	P-value †
Age								
≤75 years	1.65 (1.24, 2.21)	0.533	0.79 (0.59, 1.06)	0.495	1.26 (0.97, 1.62)	0.871	1.63 (1.25, 2.15)	0.162
>75 years	1.27 (1.12, 1.45)		0.81 (0.71, 0.92)		1.21 (1.08, 1.36)		1.39 (1.24, 1.56)	
Sex								
Female	1.45 (1.16, 1.81)	0.292	0.74 (0.60, 0.91)	0.710	1.32 (1.09, 1.60)	0.363	1.56 (1.32, 1.84)	0.254
Male	1.27 (1.11, 1.46)		0.83 (0.73, 0.96)		1.17 (1.03, 1.34)		1.32 (1.15, 1.52)	
BMI								
Normal	1.26 (1.02, 1.56)		0.75 (0.61, 0.92)		1.28 (1.04, 1.57)		1.36 (1.12, 1.64)	
Overweight	1.39 (1.11, 1.75)	0.104	0.79 (0.64, 0.99)	0.315	1.26 (1.03, 1.54)	0.384	1.48 (1.21, 1.83)	0.180
Obese	1.87 (1.31, 2.67)		0.61 (0.43, 0.85)		1.22 (0.87, 1.71)		1.66 (1.17, 2.36)	
Waist circumference								
≤97 cm	1.35 (1.13, 1.61)	0.435	0.81 (0.68, 0.97)	0.773	1.48 (1.25, 1.76)	0.173	1.36 (1.16, 1.59)	0.680
>97 cm	1.42 (1.17, 1.72)		0.71 (0.59, 0.85)		1.14 (0.96, 1.36)		1.49 (1.25, 1.78)	
Self-reported health								
Excellent/Very good/Good	1.30 (1.13, 1.49)	0.833	0.84 (0.74, 0.97)	0.185	1.22 (1.07, 1.39)	0.675	1.42 (1.24, 1.61)	0.973
Fair/Poor	1.36 (1.10, 1.69)		0.74 (0.60, 0.91)		1.26 (1.04, 1.53)		1.45 (1.20, 1.76)	

* Multivariable model adjusted for age (years), sex (male, female), race (white, non-white), enrollment site (Bowman Gray, Davis, Hopkins, Pittsburgh), education (<high school, high school, some college, college graduate), income (<$11,999, $12,000-$24,999, $25,000-$49,999, >$50,000/year), body mass index (kg/m^2), physical activity (<500, 500-1000, 1000-1500, >1500 kcal/week), waist circumference (cm), alcohol intake(0, 0-0.5, 0.5-1, 1-2, 3-7, 8-14, >14 servings/week), smoking (non-smokers, former smokers, current smokers), self-reported health (excellent/very good, good, fair/poor), and family history of cardiovascular disease (yes, no).

† P-Value obtained from continuous interaction term age, BMI, waist circumference, and categorical interaction term (females as reference) for sex, (normal BMI as reference) BMI, and (Excellent/Very good/Good health as reference) self-reported health.

Table S14. Minor fatty acids from the de novo lipogenesis pathway and the risk of all-cause mortality in the Cardiovascular Health Study: analysis of potential interaction by age, sex, body mass index, waist circumference and self-reported health, with respective stratified analyses with Bonferroni correction (significance <0.001)*

	Myristic acid (14:0)		7-hexadecenoic acid (16:1n-9)		Vaccenic acid (18:1n-7)	
	HR (95% CI)	P-value †	HR (95% CI)	P-value †	HR (95% CI)	P-value †
Age						
≤75 years	0.98 (0.71, 1.35)	0.098	1.20 (1.05, 1.38)	0.047	1.25 (0.93, 1.67)	0.232
>75 years	0.90 (0.79, 1.02)		1.11 (0.99, 1.24)		1.25 (1.12, 1.40)	
Sex						
Female	0.97 (0.79, 1.19)	0.480	1.12 (0.94, 1.33)	0.688	1.28 (1.07, 1.53)	0.866
Male	0.87 (0.76, 1.01)		1.15 (1.04, 1.28)		1.24 (1.09, 1.42)	
BMI						
Normal	0.91 (0.73, 1.13)		1.22 (1.05, 1.43)		1.23 (1.03, 1.47)	
Overweight	0.91 (0.73, 1.14)	0.011	1.09 (0.89, 1.35)	0.809	1.24 (1.00, 1.53)	0.477
Obese	0.71 (0.50, 1.01)		1.10 (0.90, 1.35)		1.54 (1.07, 2.21)	
Waist circumference						
≤97 cm	1.13 (0.94, 1.36)	0.003	1.18 (1.03, 1.35)	0.837	1.27 (1.09, 1.49)	0.636
>97 cm	0.73 (0.61, 0.88)		1.07 (0.92, 1.24)		1.37 (1.15, 1.64)	
Self-reported health						
Excellent/Very good/Good	0.89 (0.78, 1.03)	0.964	1.12 (1.00, 1.26)	0.532	1.27 (1.12, 1.45)	0.899
Fair/Poor	0.94 (0.75, 1.18)		1.18 (1.03, 1.36)		1.22 (1.02, 1.47)	

* Multivariable model adjusted for age (years), sex (male, female), race (white, non-white), enrollment site (Bowman Gray, Davis, Hopkins, Pittsburgh), education (<high school, high school, some college, college graduate), income (<$11,999, $12,000-$24,999, $25,000-$49,999, >$50,000/year), body mass index (kg/m^2), physical activity (<500, 500-1000, 1000-1500, >1500 kcal/week), waist circumference (cm), alcohol intake(0, 0-0.5, 0.5-1, 1-2, 3-7, 8-14, >14 servings/week), smoking (non-smokers, former smokers, current smokers), self-reported health (excellent/very good, good, fair/poor), and family history of cardiovascular disease (yes, no).

† P-Value obtained from continuous interaction term age, BMI, waist circumference, and categorical interaction term (females as reference) for sex, (normal BMI as reference) BMI, and (Excellent/Very good/Good health as reference) self-reported health.

Figure S1. Kaplan-Meier survival estimates for all-cause mortality by quintiles of oleic acid (18:1n-9) in the Cardiovascular Health Study.

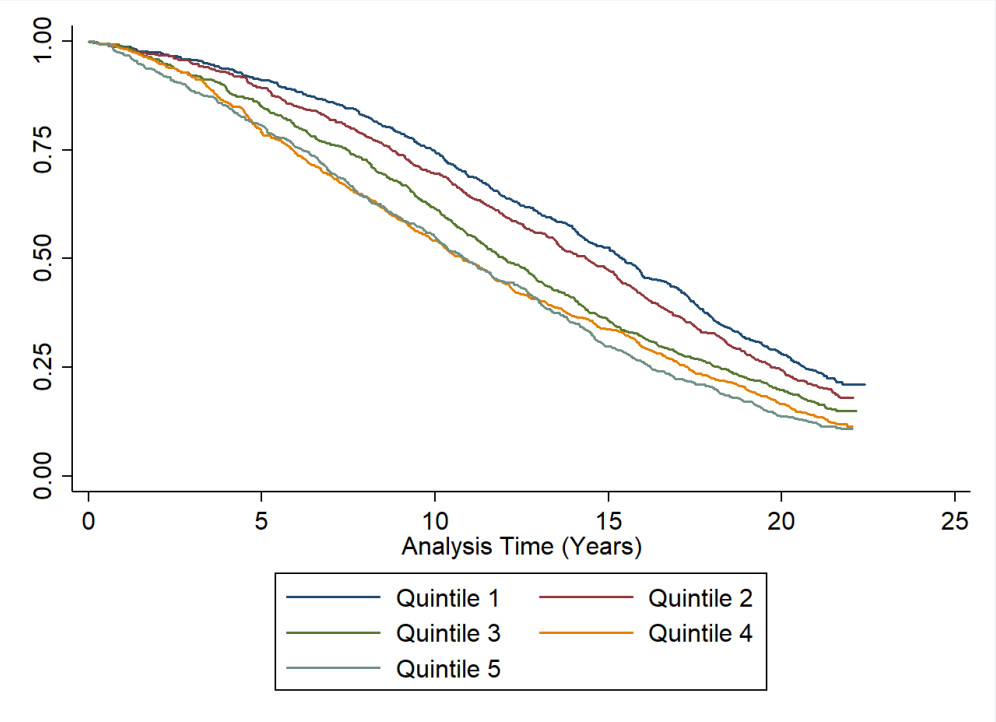
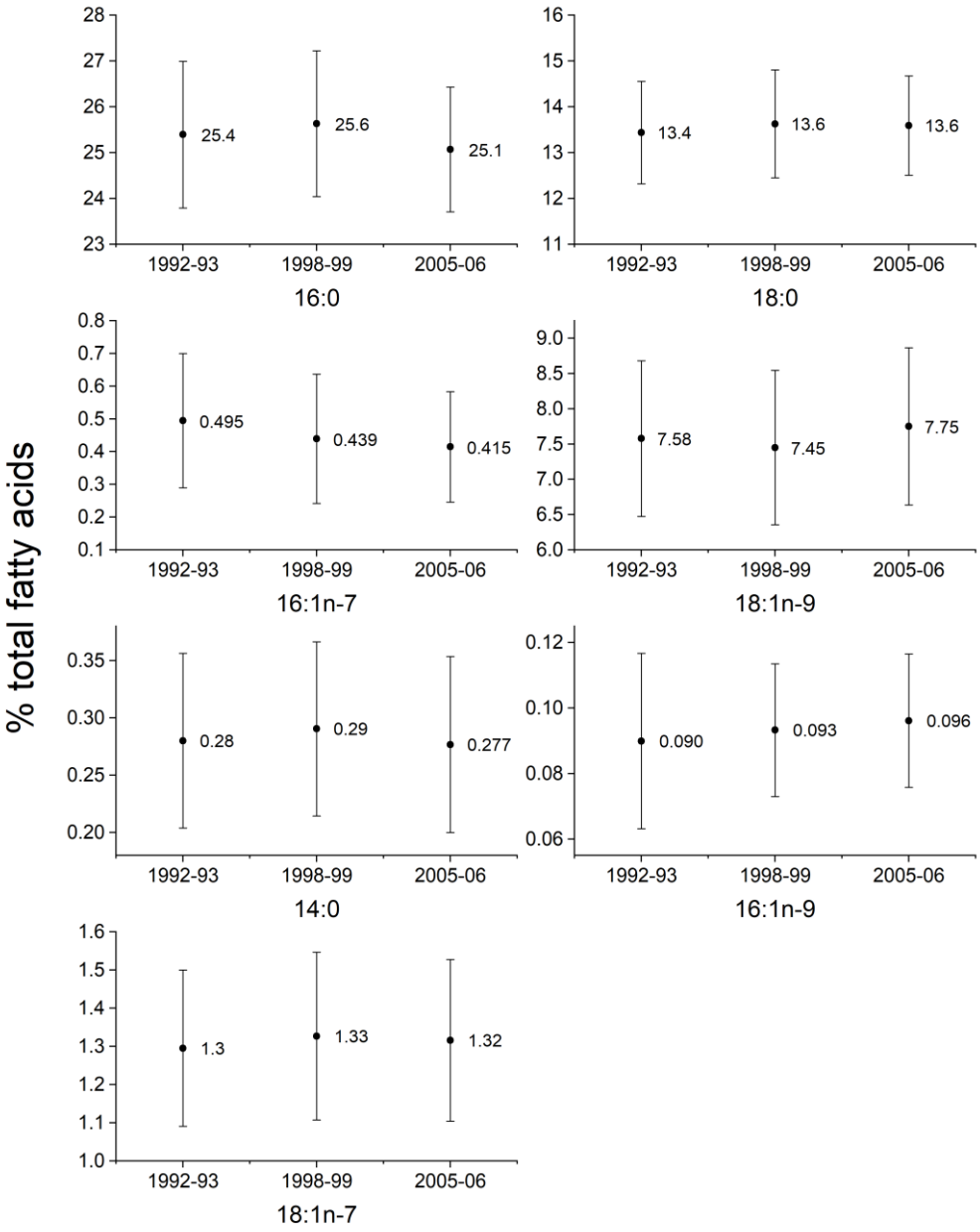
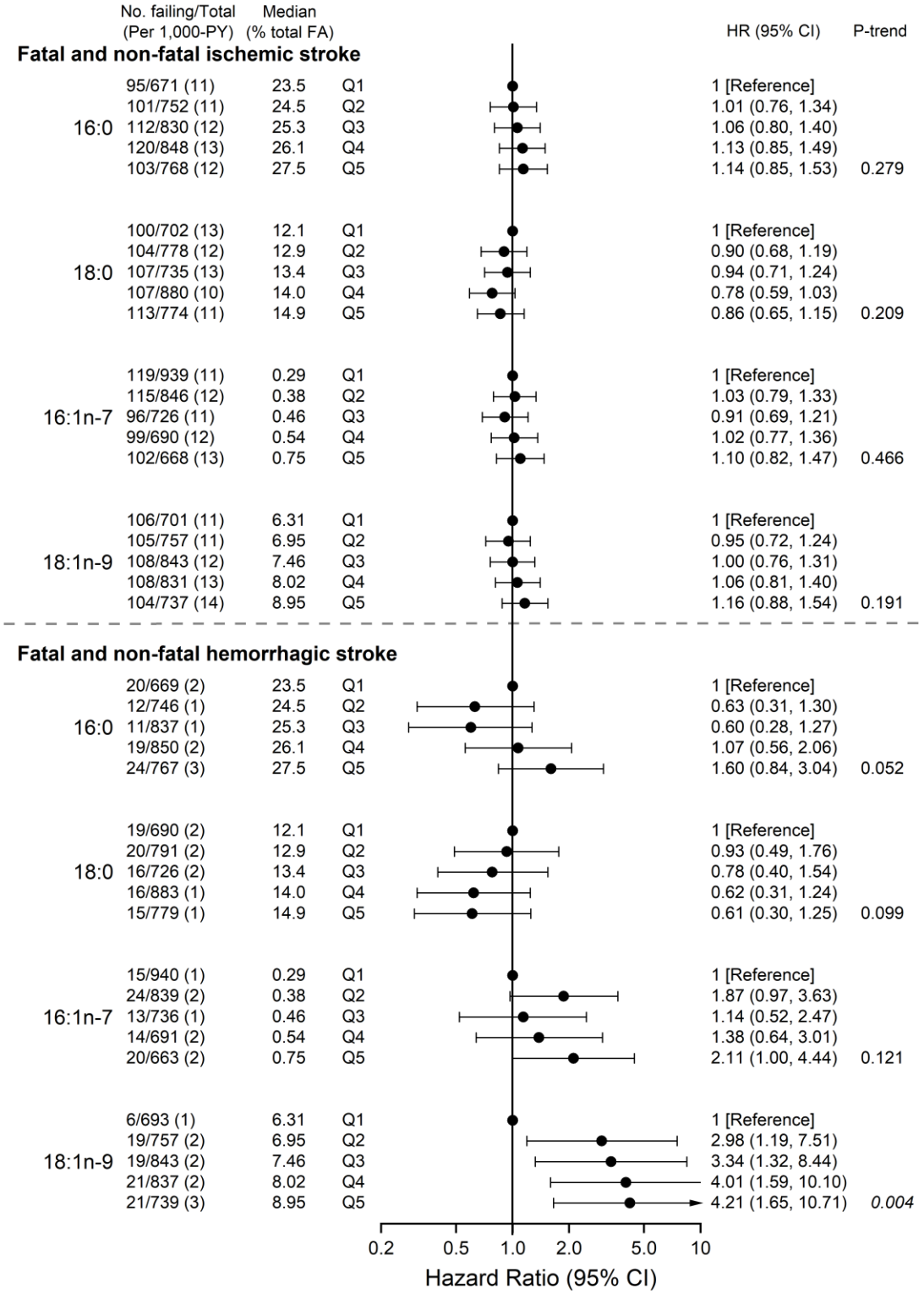


Figure S2.Mean and standard deviation of individual fatty acids in the de novo lipogenesis pathway in 1992-93 (n=3,333), 1998-99 (n=2,319), and 2005-06 (n=862) in the Cardiovascular Health Study.



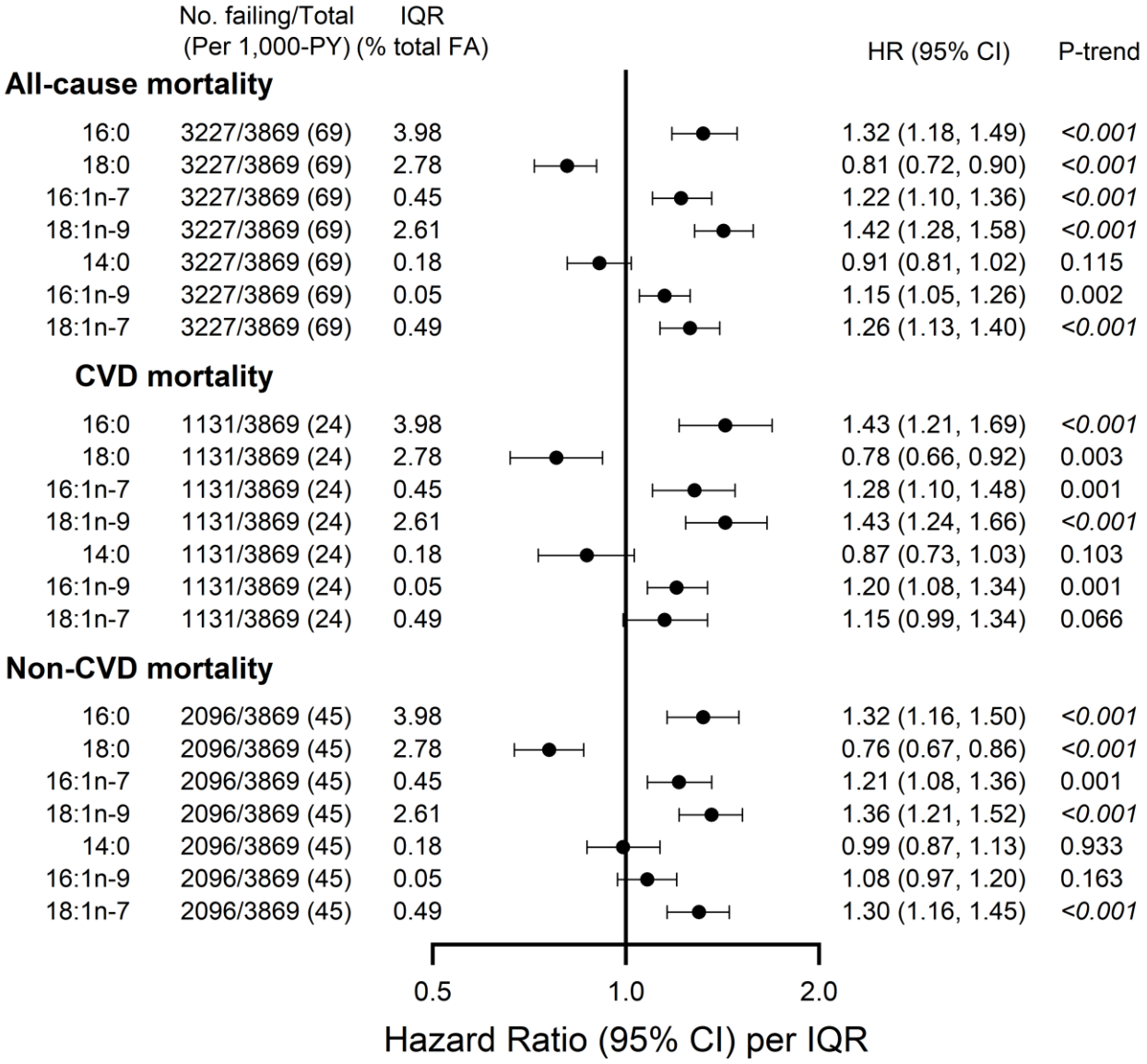
16:0 = Palmitic acid; 18:0 = Stearic acid, 16:1n-7 = Palmitoleic acid; 18:1n-9 = Oleic acid; 14:0 = Myristic acid; 16:1n-9 = 7-hexadecenoic acid; 18:1n-7 = Vaccenic acid.

Figure S3. Major fatty acids from the de novo lipogenesis pathway and the risk of fatal and non-fatal ischemic stroke and hemorrhagic stroke in the Cardiovascular Health Study after 22 years of maximum follow-up among 3,869 older adults.



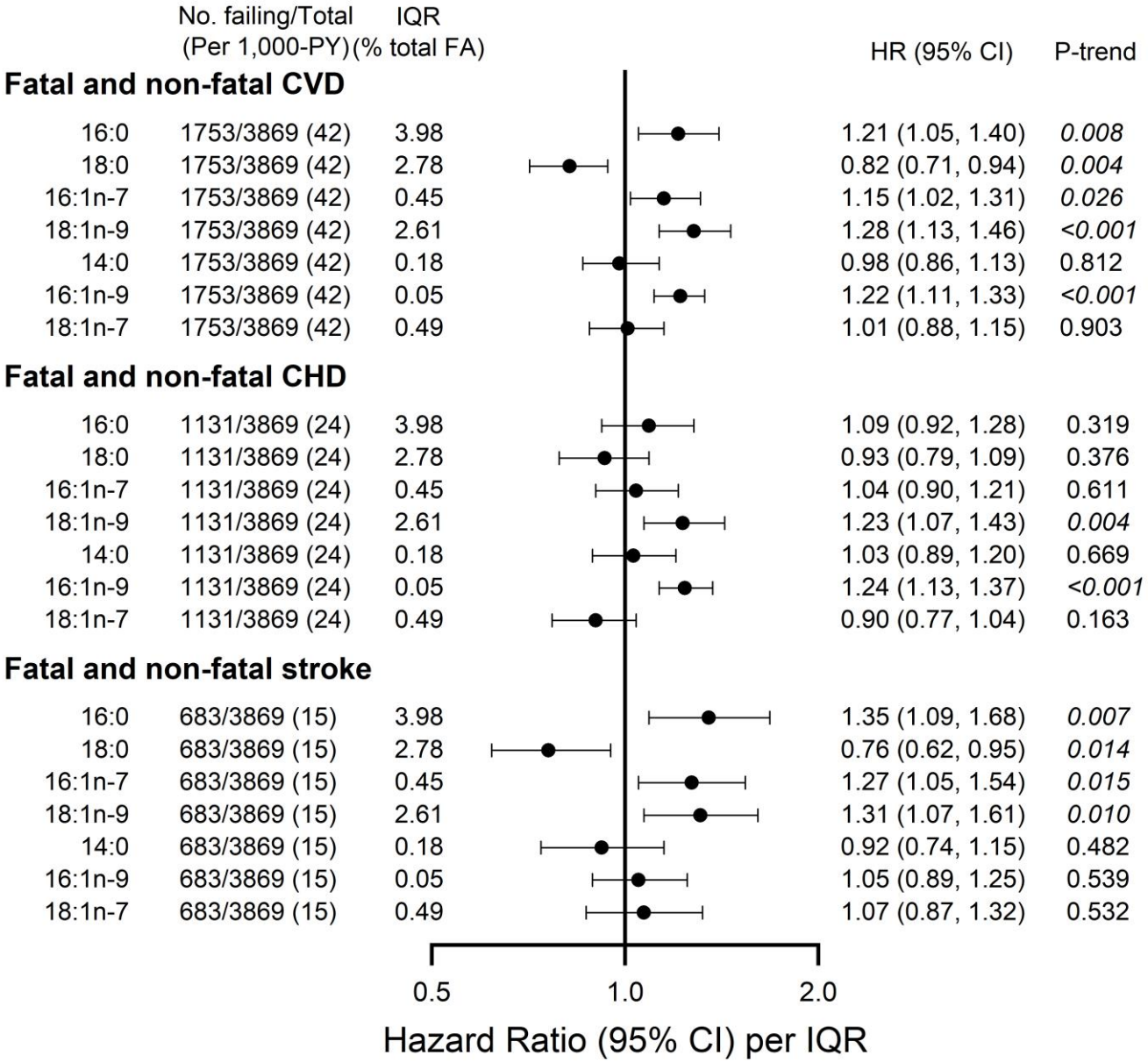
P-trend was calculated by assessing quintiles as continuous variables after assigning participants the median value in each quintile. Multivariable adjustments include age (years), sex (male, female), race (white, non-white), enrollment site (Bowman Gray, Davis, Hopkins, Pittsburgh), education (<high school, high school, some college, college graduate), income (<$11,999, $12,000-$24,999, $25,000-$49,999, >$50,000/year), body mass index (kg/m^2), physical activity (<500, 500-1000, 1000-1500, >1500 kcal/week), waist circumference (cm), alcohol intake(0, 0-0.5, 0.5-1, 1-2, 3-7, 8-14, >14 servings/week), smoking (non-smokers, former smokers, current smokers), self-reported health (excellent/very good, good, fair/poor), and family history of myocardial infarction and/or stroke (yes/no). **.** 16:0 = Palmitic acid; 18:0 = Stearic acid, 16:1n-7 = Palmitoleic acid; 18:1n-9 = Oleic acid; CI = confidence interval; FA = fatty acid; HR = hazard ratio, PY, person-years.

Figure S4. Hazard ratios (and 95% confidence intervals) of all-cause mortality, CVD mortality, and non-CVD mortality events per inter-quintile range of fatty acids from the de novo lipogenesis pathway in the Cardiovascular Health Study after 22 years of maximum follow-up among 3,869 older adults.



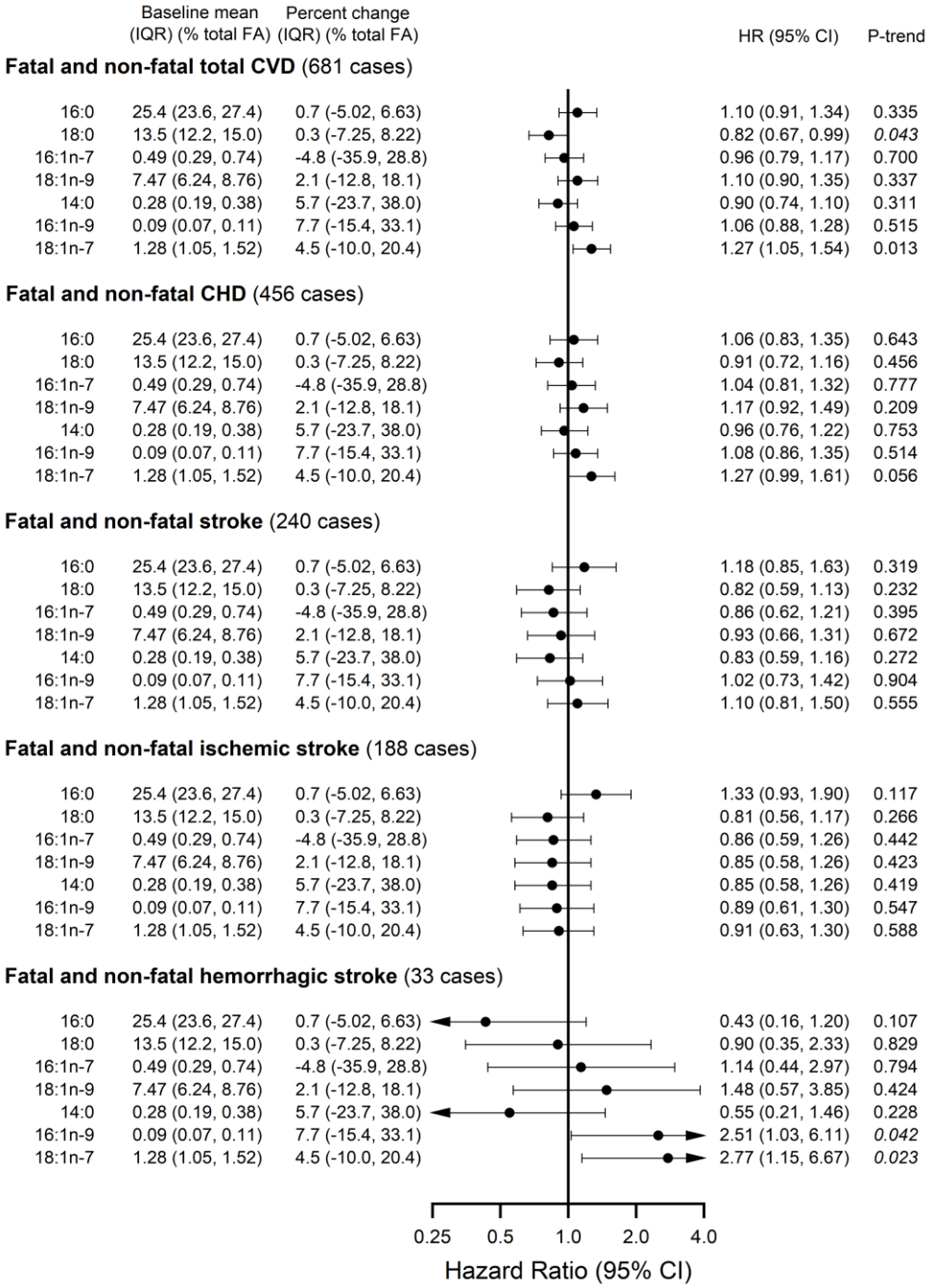
Multivariable adjustments include age (years), sex (male, female), race (white, non-white), enrollment site (Bowman Gray, Davis, Hopkins, Pittsburgh), education (<high school, high school, some college, college graduate), income (<$11,999, $12,000-$24,999, $25,000-$49,999, >$50,000/year), body mass index (kg/m2), physical activity (<500, 500-1000, 1000-1500, >1500 kcal/week), waist circumference (cm), alcohol intake(0, 0-0.5, 0.5-1, 1-2, 3-7, 8-14, >14 servings/week), smoking (non-smokers, former smokers, current smokers), self-reported health (excellent/very good, good, fair/poor), and family history of cardiovascular disease (yes, no). 16:0 = Palmitic acid; 18:0 = Stearic acid, 16:1n-7 = Palmitoleic acid; 18:1n-9 = Oleic acid; 14:0 = Myristic acid; 16:1n-9 = 7-hexadecenoic acid; 18:1n-7 = Vaccenic acid; CI = confidence interval; CVD = cardiovascular disease; FA = fatty acid; HR = hazard ratio; PY, person-years.

Figure S5. Hazard ratios (and 95% confidence intervals) of fatal and non-fatal total CVD, fatal and non-fatal CHD, and fatal and non-fatal stroke events per inter-quintile range of major fatty acids from the de novo lipogenesis pathway in the Cardiovascular Health Study after 22 years of maximum follow-up among 3,869 older adults.



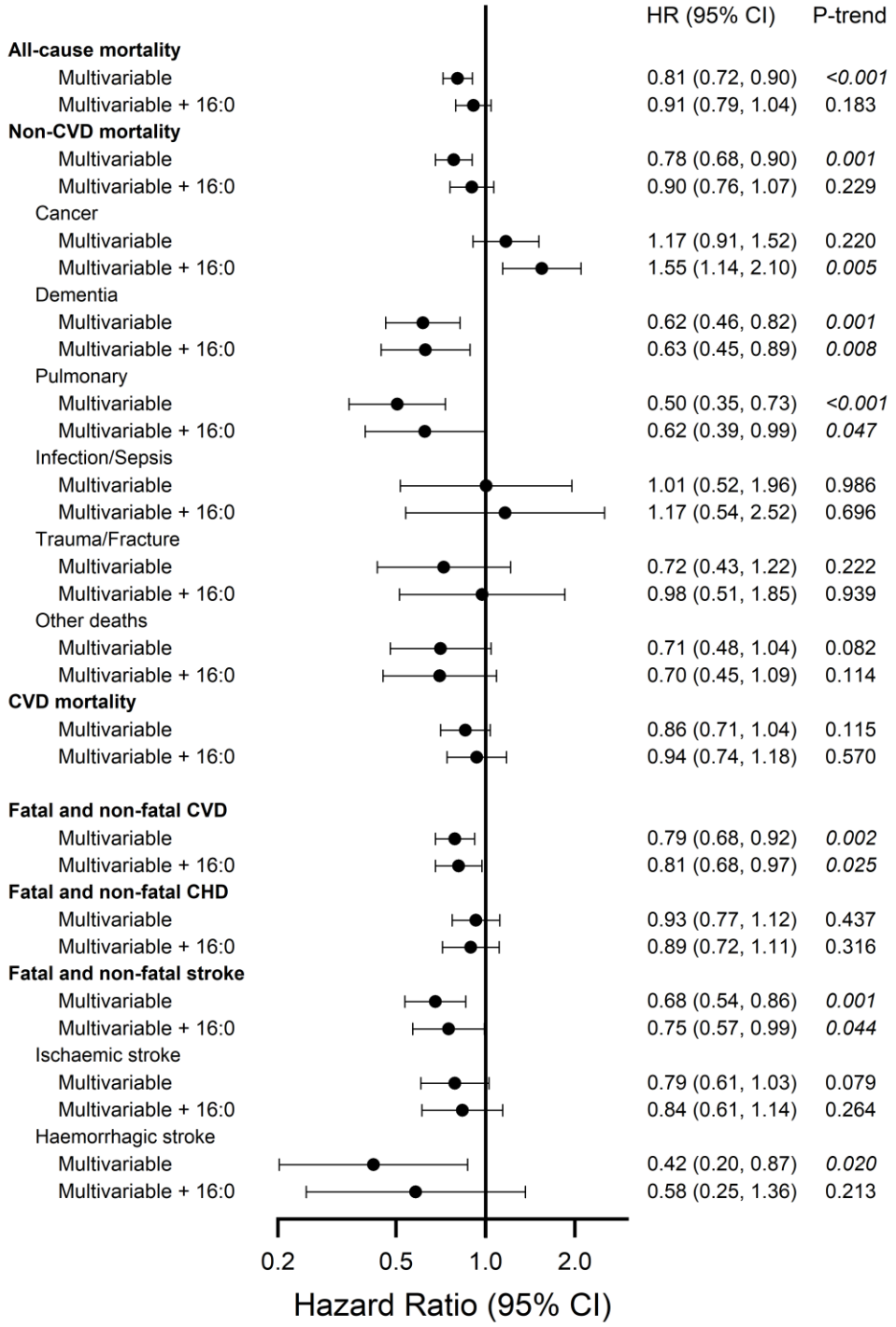
Multivariable adjustments include age (years), sex (male, female), race (white, non-white), enrollment site (Bowman Gray, Davis, Hopkins, Pittsburgh), education (<high school, high school, some college, college graduate), income (<$11,999, $12,000-$24,999, $25,000-$49,999, >$50,000/year), body mass index (kg/m2), physical activity (<500, 500-1000, 1000-1500, >1500 kcal/week), waist circumference (cm), alcohol intake(0, 0-0.5, 0.5-1, 1-2, 3-7, 8-14, >14 servings/week), smoking (non-smokers, former smokers, current smokers), self-reported health (excellent/very good, good, fair/poor), and family history of cardiovascular disease (yes, no). **.** 16:0 = Palmitic acid; 18:0 = Stearic acid, 16:1n-7 = Palmitoleic acid; 18:1n-9 = Oleic acid; 14:0 = Myristic acid; 16:1n-9 = 7-hexadecenoic acid; 18:1n-7 = Vaccenic acid; CHD = coronary heart disease; CI = confidence interval; CVD = cardiovascular disease; FA = fatty acid; HR = hazard ratio; PY, person-years.

Figure S6. Hazard ratios (and 95% confidence intervals) of fatal and non-fatal total CVD and CVD subtypes per inter-quintile range (IQR) of percent change for fatty acids from the de novo lipogenesis pathway in the Cardiovascular Health Study after 16 years of maximum follow-up among 1,815 older adults.



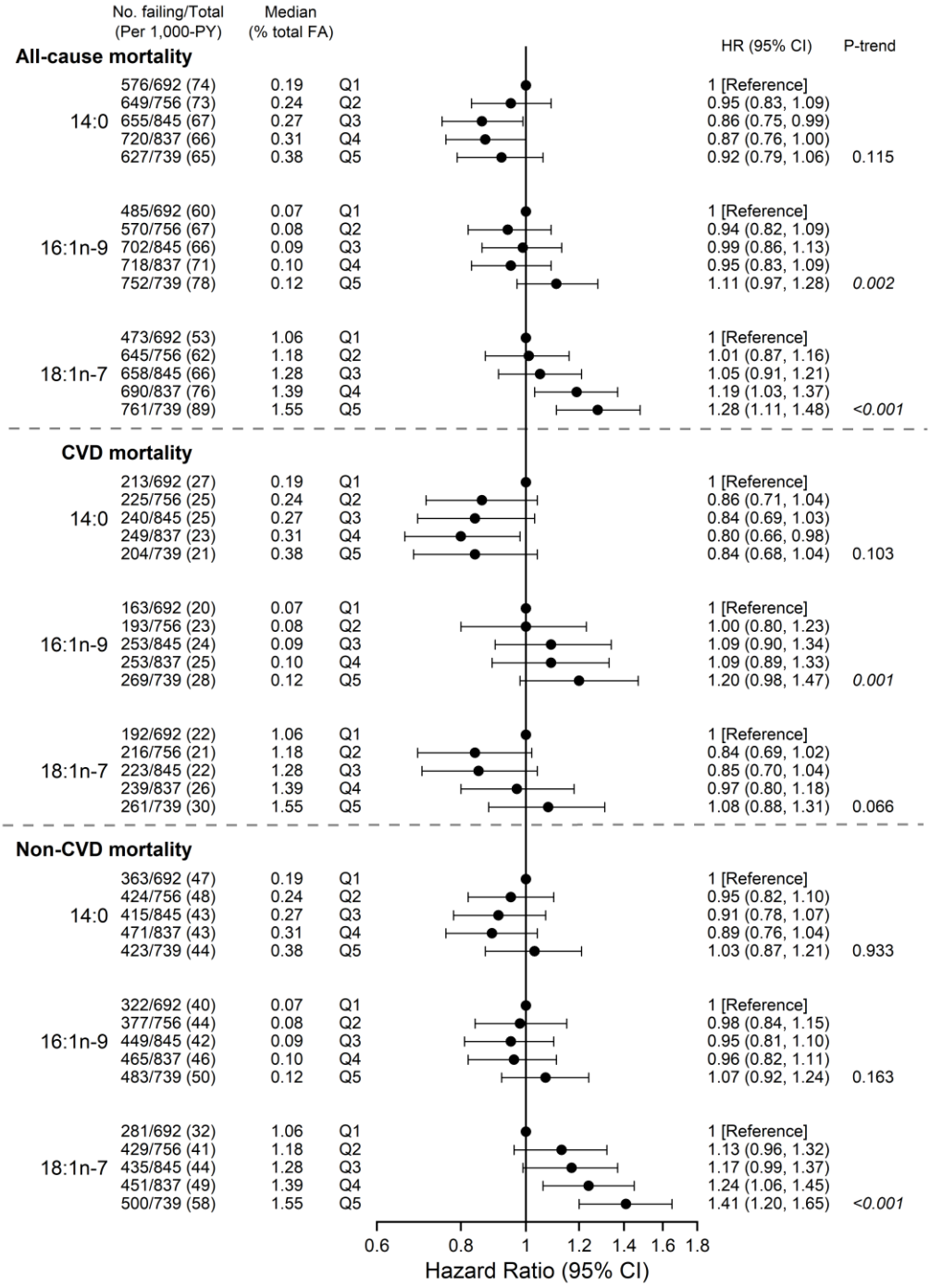
The IQR is estimated to be the difference between the midpoint of the 1st and 5th quintile. Baseline (IQR), expressed in % total fatty acids, represents the fatty acid levels at study baseline in 1992-93. Percent change (IQR), is the mean of changes between 1992-93 and 1998-99, and 1998-99 and 2005-06. Multivariable, diet-adjustments additionally include race (white, non-white), enrollment site (Bowman Gray, Davis, Hopkins, Pittsburgh), education (<high school, high school, some college, college graduate), income (<$11,999, $12,000-$24,999, $25,000-$49,999, >$50,000/year), body mass index (kg/m2), physical activity (<500, 500-1000, 1000-1500, >1500 kcal/week), waist circumference (cm), alcohol intake(0, 0-0.5, 0.5-1, 1-2, 3-7, 8-14, >14 servings/week), smoking (non-smokers, former smokers, current smokers), self-reported health (excellent/very good, good, fair/poor), and family history of cardiovascular disease (yes, no). 16:0 = Palmitic acid; 18:0 = Stearic acid, 16:1n-7 = Palmitoleic acid; 18:1n-9 = Oleic acid; 14:0 = Myristic acid; 16:1n-9 = 7-hexadecenoic acid; 18:1n-7 = Vaccenic acid; CHD = coronary heart disease; CI = confidence interval; CVD = cardiovascular disease; FA = fatty acid; HR = hazard ratio; IQR = interquintile range.

Figure S7 Hazard ratios and 95% confidence intervals of serial cumulative plasma phospholipid stearic acid levels (18:0) per inter-quintile range and risk of all-cause mortality, CVD mortality, non-CVD mortality and subtypes, and incident CVD and subtypes, with and without adjustment for serial cumulative levels of palmitic acid (16:0).



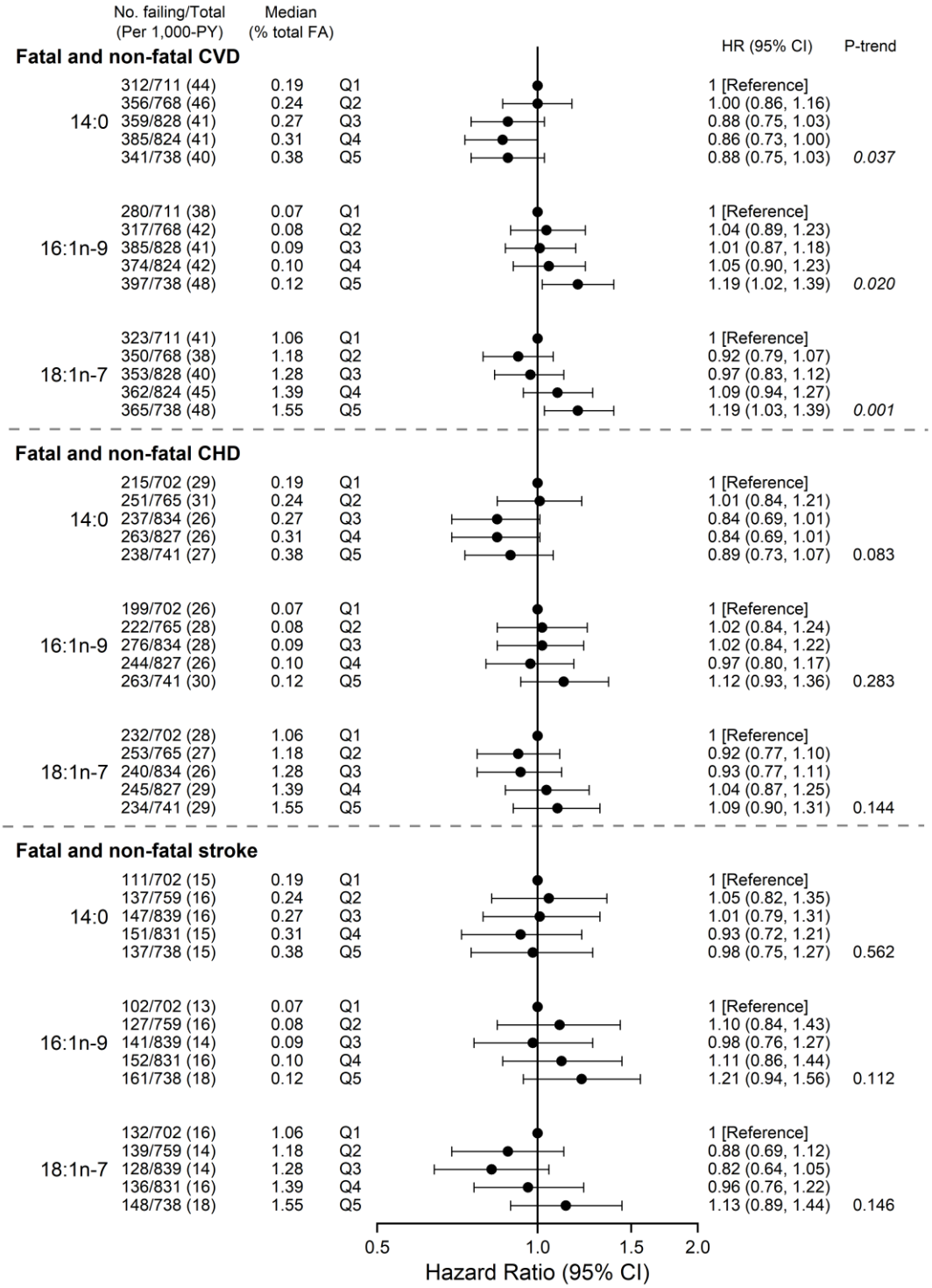
Multivariable adjustments include age (years), sex (male, female), race (white, non-white), enrollment site (Bowman Gray, Davis, Hopkins, Pittsburgh), education (<high school, high school, some college, college graduate), income (<$11,999, $12,000-$24,999, $25,000-$49,999, >$50,000/year), body mass index (kg/m2), physical activity (<500, 500-1000, 1000-1500, >1500 kcal/week), waist circumference (cm), alcohol intake(0, 0-0.5, 0.5-1, 1-2, 3-7, 8-14, >14 servings/week), smoking (non-smokers, former smokers, current smokers), self-reported health (excellent/very good, good, fair/poor), and family history of cardiovascular disease (yes, no). 16:0 = Palmitic acid; CHD = coronary heart disease; CI = confidence interval; CVD = cardiovascular disease; HR = hazard ratio.

Figure S8. Minor fatty acids from the de novo lipogenesis pathway and the risk all-cause mortality, cardiovascular mortality, and non-cardiovascular mortality in the Cardiovascular Health Study after 22 years of maximum follow-up among 3,869 older adults.



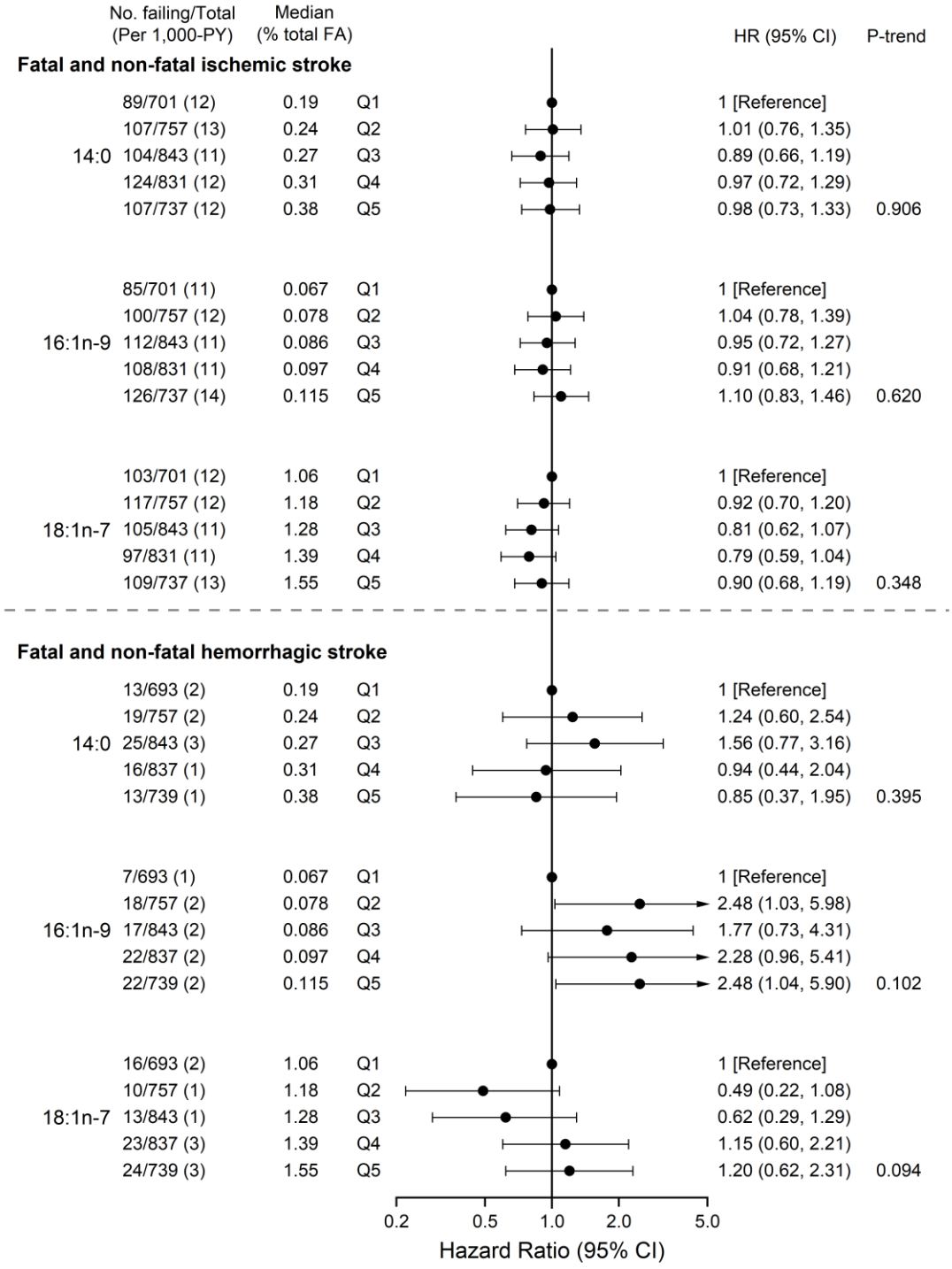
P-trend was calculated by assessing quintiles as continuous variables after assigning participants the median value in each quintile. Multivariable adjustments include age (years), sex (male, female), race (white, non-white), enrollment site (Bowman Gray, Davis, Hopkins, Pittsburgh), education (<high school, high school, some college, college graduate), income (<$11,999, $12,000-$24,999, $25,000-$49,999, >$50,000/year), body mass index (kg/m^2), physical activity (<500, 500-1000, 1000-1500, >1500 kcal/week), waist circumference (cm), alcohol intake(0, 0-0.5, 0.5-1, 1-2, 3-7, 8-14, >14 servings/week), smoking (non-smokers, former smokers, current smokers), self-reported health (excellent/very good, good, fair/poor), and family history of myocardial infarction and/or stroke (yes/no). 14:0 = Myristic acid; 16:1n-9 = 7-hexadecenoic acid; 18:1n-7 = Vaccenic acid; CI = confidence interval; CVD = cardiovascular disease; FA = fatty acid; HR = hazard ratio; PY, person-years.

Figure S9. Minor fatty acids from the de novo lipogenesis pathway and the risk of fatal and non-fatal cardiovascular disease, coronary heart disease (CHD) and stroke in the Cardiovascular Health Study after 22 years of maximum follow-up among 3,869 older adults.



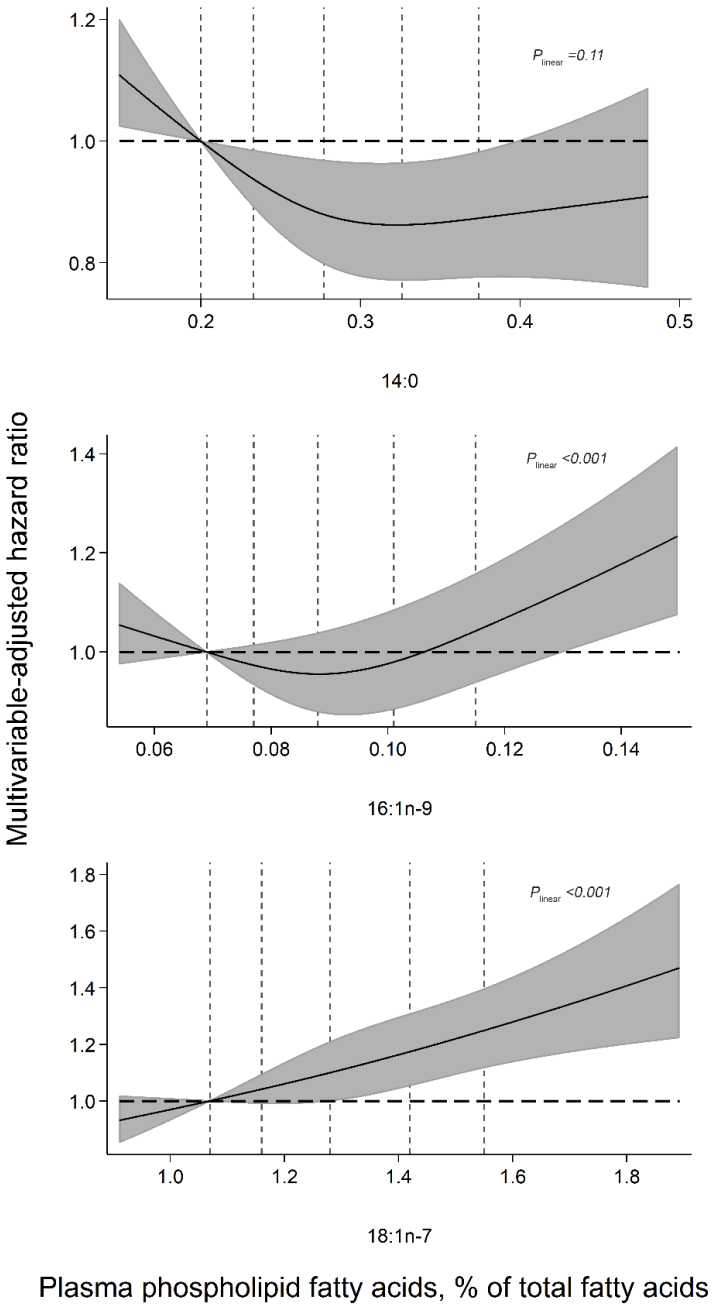
P-trend was calculated by assessing quintiles as continuous variables after assigning participants the median value in each quintile. Multivariable adjustments include age (years), sex (male, female), race (white, non-white), enrollment site (Bowman Gray, Davis, Hopkins, Pittsburgh), education (<high school, high school, some college, college graduate), income (<$11,999, $12,000-$24,999, $25,000-$49,999, >$50,000/year), body mass index (kg/m^2), physical activity (<500, 500-1000, 1000-1500, >1500 kcal/week), waist circumference (cm), alcohol intake(0, 0-0.5, 0.5-1, 1-2, 3-7, 8-14, >14 servings/week), smoking (non-smokers, former smokers, current smokers), self-reported health (excellent/very good, good, fair/poor), family history of myocardial infarction and/or stroke (yes/no). 14:0 = Myristic acid; 16:1n-9 = 7-hexadecenoic acid; 18:1n-7 = Vaccenic acid; CI = confidence interval; FA = fatty acid; HR = hazard ratio; PY, person-years.

Figure S10. Minor fatty acids from the de novo lipogenesis pathway and the risk of fatal and non-fatal ischemic stroke and hemorrhagic stroke in the Cardiovascular Health Study after 22 years of maximum follow-up among 3,869 older adults.



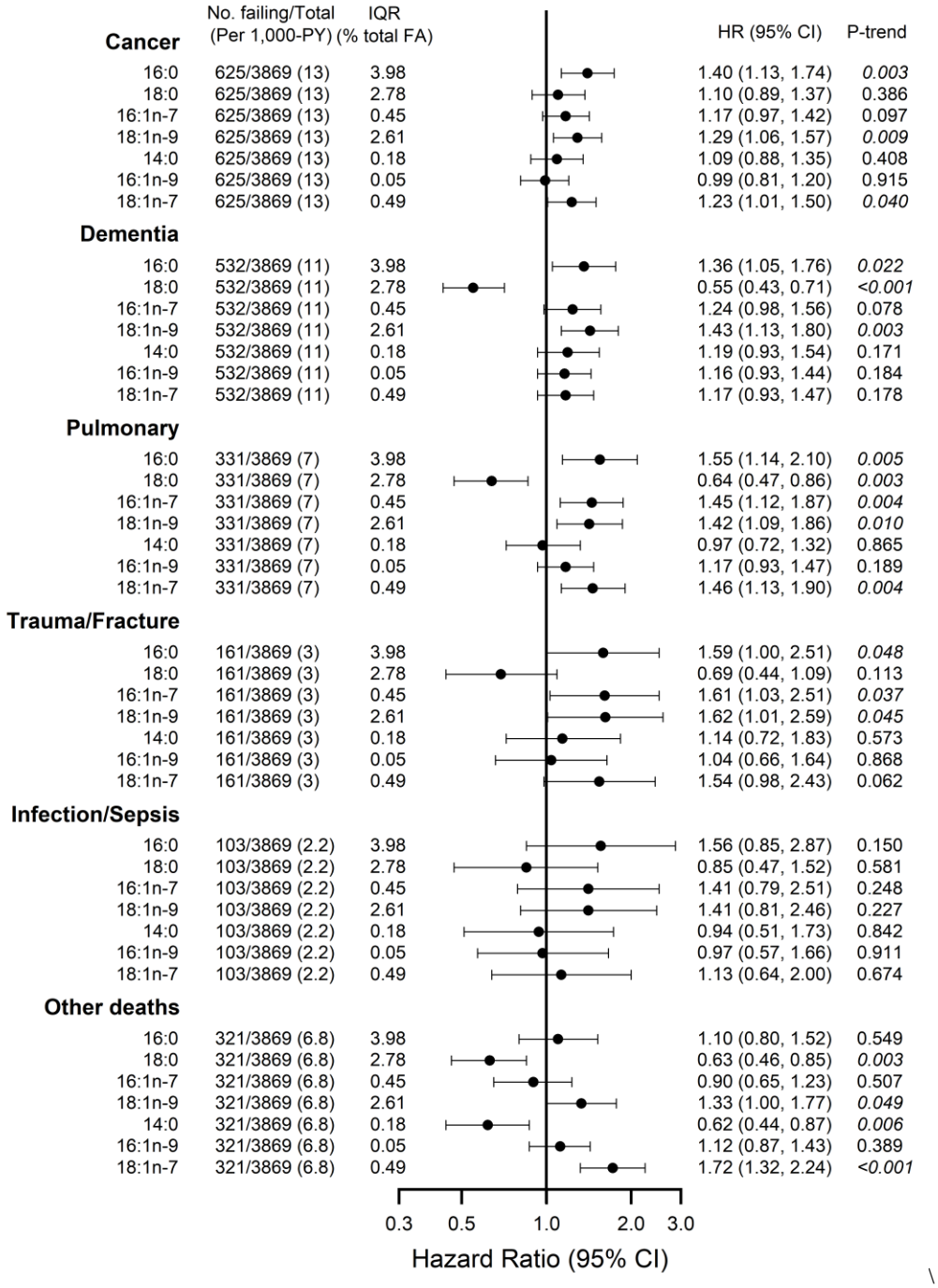
P-trend was calculated by assessing quintiles as continuous variables after assigning participants the median value in each quintile. Multivariable adjustments include age (years), sex (male, female), race (white, non-white), enrollment site (Bowman Gray, Davis, Hopkins, Pittsburgh), education (<high school, high school, some college, college graduate), income (<$11,999, $12,000-$24,999, $25,000-$49,999, >$50,000/year), body mass index (kg/m^2), physical activity (<500, 500-1000, 1000-1500, >1500 kcal/week), waist circumference (cm), alcohol intake(0, 0-0.5, 0.5-1, 1-2, 3-7, 8-14, >14 servings/week), smoking (non-smokers, former smokers, current smokers), self-reported health (excellent/very good, good, fair/poor), family history of myocardial infarction and/or stroke (yes/no). 14:0 = Myristic acid; 16:1n-9 = 7-hexadecenoic acid; 18:1n-7 = Vaccenic acid; CI = confidence interval; FA = fatty acid; HR = hazard ratio; PY, person-years.

Figure S11. Multivariable-adjusted relationship of minor cumulative plasma phospholipid fatty acids from the de novo lipogenesis pathway with risk of all-cause mortality, evaluated using restricted cubic splines.



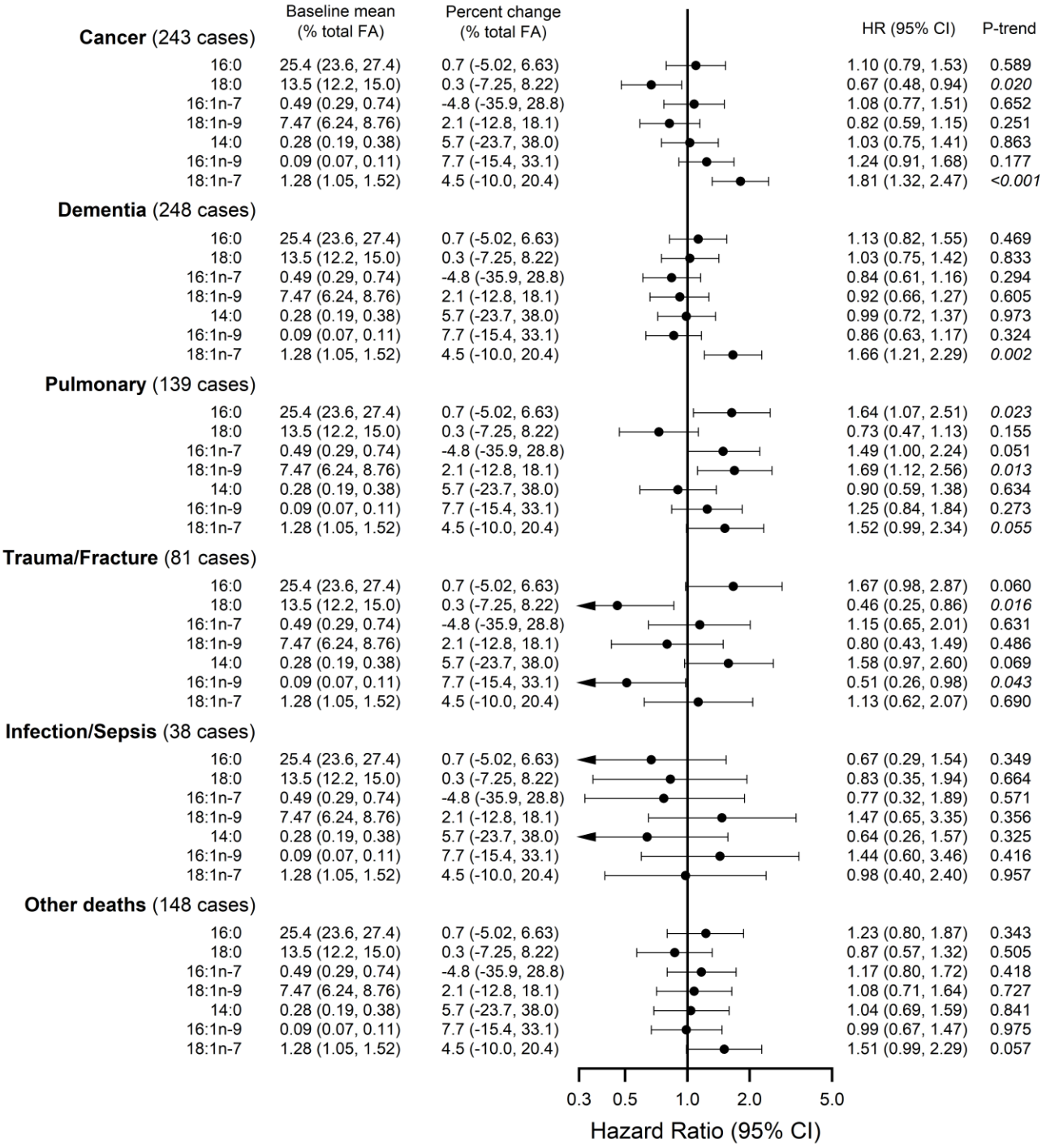
The solid lines and shaded area represents the central risk estimate and 95% confidence interval, respectively, for each fatty acid. The dotted vertical lines correspond to the 10th, 25th, 50th, 75th, and 90th percentiles for each fatty acid. The top and bottom 1% of participants were omitted as outliers to provide better visualization. Evidence for non-linearity was calculated by performing a likelihood ratio test between a multivariable model with all spline terms versus a multivariable model with only the linear term. No evidence for non-linearity was found for 18:1n-7 where P_{curve}=0.94; for 14:0 and 16:1n-9, P_{curve}=0.04 and P_{curve}=0.01 respectively, suggesting a possible U-shaped effect. Multivariable-adjustments include age (years), sex (male, female), race (white, non-white), enrollment site (Bowman Gray, Davis, Hopkins, Pittsburgh), education (<high school, high school, some college, college graduate), income (<$11,999, $12,000-$24,999, $25,000-$49,999, >$50,000/year), body mass index (kg/m^2), physical activity (<500, 500-1000, 1000-1500, >1500 kcal/week), waist circumference (cm), alcohol intake(0, 0-0.5, 0.5-1, 1-2, 3-7, 8-14, >14 servings/week), smoking (non-smokers, former smokers, current smokers), self-reported health (excellent/very good, good, fair/poor), and family history of cardiovascular disease (yes, no). 14:0 = Myristic acid; 16:1n-9 = 7-hexadecenoic acid; 18:1n-7 = Vaccenic acid.

Figure S12. Hazard ratios (and 95% confidence intervals) of cause-specific non-cardiovascular mortality events per inter-quintile range of major fatty acids from the de novo lipogenesis pathway in the Cardiovascular Health Study after 22 years of maximum follow-up among 3,869 older adults.



Multivariable adjustments include age (years), sex (male, female), race (white, non-white), enrollment site (Bowman Gray, Davis, Hopkins, Pittsburgh), education (<high school, high school, some college, college graduate), income (<$11,999, $12,000-$24,999, $25,000-$49,999, >$50,000/year), body mass index (kg/m^2), physical activity (<500, 500-1000, 1000-1500, >1500 kcal/week), waist circumference (cm), alcohol intake(0, 0-0.5, 0.5-1, 1-2, 3-7, 8-14, >14 servings/week), smoking (non-smokers, former smokers, current smokers), self-reported health (excellent/very good, good, fair/poor), and family history of cardiovascular disease (yes, no). 16:0 = Palmitic acid; 18:0 = Stearic acid, 16:1n-7 = Palmitoleic acid; 18:1n-9 = Oleic acid; 14:0 = Myristic acid; 16:1n-9 = 7-hexadecenoic acid; 18:1n-7 = Vaccenic acid; CI = confidence interval; FA = fatty acid; HR = hazard ratio; PY; person-years.

Figure S13. Hazard ratios (and 95% confidence intervals) of non-CVD mortality subtypes per inter-quintile range (IQR) of percent change for fatty acids from the de novo lipogenesis pathway in the Cardiovascular Health Study after 16 years of maximum follow-up among 1,815 older adults.



The IQR is estimated to be the difference between the midpoint of the 1st and 5th quintile. Baseline (IQR), expressed in % total fatty acids, represents the fatty acid levels at study baseline in 1992-93. Percent change (IQR), is the mean of changes between 1992-93 and 1998-99, and 1998-99 and 2005-06. Multivariable, diet-adjustments additionally include race (white, non-white), enrollment site (Bowman Gray, Davis, Hopkins, Pittsburgh), education (<high school, high school, some college, college graduate), income (<$11,999, $12,000-$24,999, $25,000-$49,999, >$50,000/year), body mass index (kg/m2), physical activity (<500, 500-1000, 1000-1500, >1500 kcal/week), waist circumference (cm), alcohol intake(0, 0-0.5, 0.5-1, 1-2, 3-7, 8-14, >14 servings/week), smoking (non-smokers, former smokers, current smokers), self-reported health (excellent/very good, good, fair/poor), and family history of cardiovascular disease (yes, no). 16:0 = Palmitic acid; 18:0 = Stearic acid, 16:1n-7 = Palmitoleic acid; 18:1n-9 = Oleic acid; 14:0 = Myristic acid; 16:1n-9 = 7-hexadecenoic acid; 18:1n-7 = Vaccenic acid; CI = confidence interval; FA = fatty acid; HR = hazard ratio; IQR = interquintile range.