ORIGINAL RESEARCH

Serial Biomarkers of De Novo Lipogenesis Fatty Acids and Incident Heart Failure in Older Adults: The Cardiovascular Health Study

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***Background*—**De novo lipogenesis (DNL) is an endogenous pathway that converts excess dietary starch, sugar, protein, and alcohol into specific fatty acids (FAs). Although elevated DNL is linked to several metabolic abnormalities, little is known about how long-term habitual levels and changes in levels of FAs in the DNL pathway relate to incident heart failure (HF).

***Methods and Results*—**We investigated whether habitual levels and changes in serial measures of FAs in the DNL pathway were associated with incident HF among 4249 participants free of HF at baseline. Plasma phospholipid FAs were measured at baseline, 6 years, and 13 years using gas chromatography, and risk factors for HF were measured using standardized methods. Incident HF was centrally adjudicated using medical records. We prospectively evaluated associations with HF risk of (1) habitual FA levels, using cumulative updating to assess long-term exposure, and (2) changes in FA levels over time. During 22.1 years of follow-up, 1304 HF cases occurred. After multivariable adjustment, habitual levels and changes in levels of palmitic acid (16:0) were positively associated with incident HF (interquintile hazard ratio [95% CI]=1.17 [1.00-1.36] and 1.26 [1.03-1.55], respectively). Changes in levels of 7-hexadecenoic acid (16:1n-9) and vaccenic acid (18:1n-7) were each positively associated with risk of HF (1.36 [1.13-1.62], and 1.43 [1.18-1.72], respectively). Habitual levels and changes in levels of myristic acid (14:0), palmitoleic acid (16:1n-7), stearic acid (18:0), and oleic acid (18:1n-9) were not associated with incident HF.

***Conclusions*—**Both habitual levels and changes in levels of 16:0 were positively associated with incident HF in older adults. Changes in 16:1n-9 and 18:1n-7 were also positively associated with incident HF. These findings support a potential role of DNL or these DNL-related FAs in the development of HF. **(*J Am Heart Assoc*. 2020;9:e014119. DOI: 10.1161/JAHA.119.014119.)**

Key Words: de novo lipogenesis • fatty acids • heart failure • left ventricular ejection fraction

Heart failure (HF) is a growing clinical and public health problem, accounting for 1 in 8 deaths in the United States.[1] By 2030, more than 8 million Americans are expected to have HF.[2] The prevalence of HF increases substantially with age,[1] and about 3 in 4 hospitalizations for HF occur in individuals aged 65 years and older.[3] HF is associated with high morbidity and mortality; half of Medicare beneficiaries hospitalized with HF survive <3 years.[4] Given these large and growing burdens, it is crucial to identify novel risk factors that may be leveraged for primary prevention and novel treatments for HF.

Obesity and diabetes mellitus are each a risk factor for HF. One of the most striking abnormalities associated with these risk factors is de novo lipogenesis (DNL), an endogenous pathway in the liver that converts excess dietary starch, sugar, protein, and alcohol into fatty acids.[5,6] Although hepatic DNL is tightly regulated in healthy individuals,[7] elevated DNL appears to be a key driver of hepatic steatosis and is commonly observed in people with visceral obesity, diabetes mellitus, nonalcoholic fatty liver disease (NAFLD), and metabolic syndrome.[8,9] Accordingly, higher DNL is associated with HF risk factors including insulin resistance, diabetes mellitus, hypertension, inflammation, atherogenic dyslipidemia, and visceral adiposity.[10-12] In addition, growing

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Accompanying Tables S1 through S7 and Figures S1 through S5 are available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.119.014119

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Clinical Perspective

What Is New?

- Elevated hepatic de novo lipogenesis (DNL)—by which the liver synthesizes fatty acids from dietary starch, sugar, protein, or alcohol—is 1 of the earliest metabolic abnormalities related to fatty liver, insulin resistance, and metabolic syndrome, yet the relationship of DNL with new heart failure is not well established.
- We investigated whether circulating biomarker levels of fatty acids in the DNL pathway are associated with onset of heart failure in older US adults.
- Both habitual levels and changes in levels over time of palmitic acid (a 16-carbon saturated fat) were positively associated with incident heart failure, and changes in levels of 2 other DNL fatty acids—7-hexadecenoic acid and vaccenic acid—also were positively associated with incident heart failure.

What Are the Clinical Implications?

- Higher habitual levels and increases in levels of specific fatty acids in the DNL pathway were independently linked to increased risk of heart failure.
- If these associations prove to be causal, DNL and its specific fatty acid products could be targeted to reduce risk of heart failure, for example, by minimizing dietary refined starch, sugars, and alcohol or through novel molecular interventions.

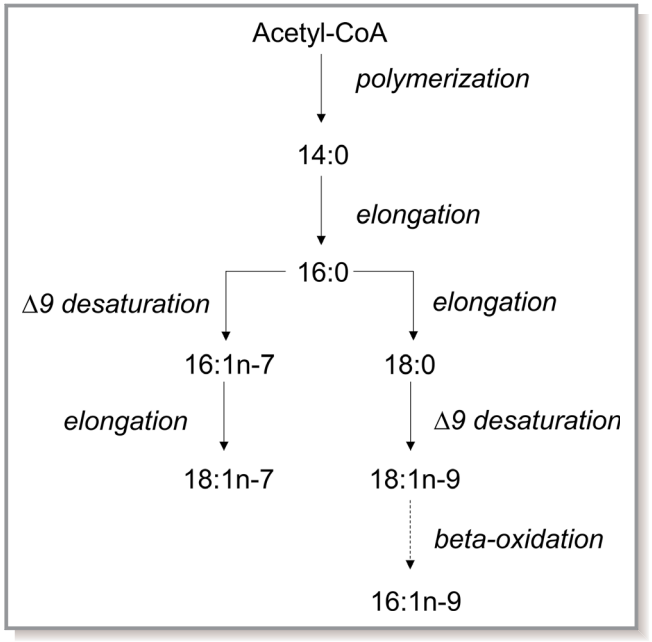

Figure 1. Plasma phospholipid fatty acids produced in de novo lipogenesis. Acetyl-coenzyme A (acetyl-CoA) is polymerized to form fatty acids. The initial major product of de novo lipogenesis is palmitic acid (16:0), which can be converted to palmitoleic acid (16:1n-7) by Δ9 desaturation or stearic acid (18:0) by elongation. These fatty acids can be further converted to vaccenic acid (18:1n-7) and oleic acid (18:1n-9). Myristic acid (14:0) is another possible minor product of fatty acid synthesis. 7-Hexadecenoic acid (16:1n-9) could arise from the β-oxidation of 18:1n-9 in cell culture studies.[15] The regular arrows indicate fatty acid biosynthesis, and the dashed arrow indicates β-oxidation, which is not a part of biosynthesis.

evidence suggests that NAFLD is associated with altered cardiac structure and left ventricular diastolic dysfunction, increasing the risk of HF.[13,14]

The major pathway for DNL involves conversion of acetyl-coA into palmitic acid (16:0), which can then be elongated and/or desaturated to stearic acid (18:0), palmitoleic acid (16:1n-7), vaccenic acid (18:1n-7), and oleic acid (18:1n-9) (Figure 1). Additional minor fatty acids synthesized in the DNL pathway include myristic acid (14:0) and possibly 7-hexadecenoic acid (16:1n-9).[15] Although direct hepatic DNL synthesis can be measured using stable isotope administration (ie, oral intake of heavy water or ^{13}C-acetate),[9,16] labeling studies are far too costly and labor intensive for large studies. In prior interventional and observational studies, blood levels of circulating fatty acids in the DNL pathway have been used as biomarkers of DNL.[17-19] Our prior work showed that circulating levels of these fatty acids were weakly correlated with their direct dietary intake, consistent with the importance of endogenous synthesis.[20] Consistent with this, in prior interventional studies low fat, high carbohydrate diets increased blood levels of most of fatty acids in the DNL pathway, especially 16:0 and 16:1n-7, within weeks.[19,21,22]

In addition to its effects on hepatic steatosis, DNL may influence HF through direct effects of the fatty acids in the DNL pathway. Several of these metabolites have significant biological activity. In animal and in vitro studies, 16:0 and 18:0 induced proinflammatory pathways, endoplasmic reticulum stress, cellular apoptosis, and insulin resistance.[23-32] In contrast, when exogenously provided or produced outside the liver, 16:1n-7 may have protective effects against insulin sensitivity and inflammation,[33] whereas 18:1n-9 may reduce insulin resistance and prevent apoptosis.[34,35] Experimental evidence for other fatty acids in the DNL pathway has been mixed.[33,36-40]

Thus, elevated circulating fatty acids in the DNL pathway may mark underlying activation of DNL, a contributor to hepatic steatosis, visceral adiposity, and related metabolic risk factors for HF and may have intrinsic biological effects that could increase risk of HF. Yet, relations of fatty acids in the DNL pathway with HF and HF subtypes are not well established. Relatively few studies have investigated associations of these fatty acids with HF,[41-45] especially among older age when risk is highest. In addition, prior biomarker

studies obtained only a single measurement of fatty acids of baseline, which leads to misclassification and regression dilution bias due to changes in levels over time. No studies have evaluated serial measures to assess how habitual levels as well as changes in levels of these fatty acid biomarkers relate to risk of HF. Finally, associations of these fatty acids with risk of HF with and without left ventricular systolic or diastolic dysfunction have not been explored. To address these gaps in knowledge, we prospectively investigated associations of serial biomarker measures of fatty acids in the DNL pathway with incident HF and HF subtypes in older adults in the CHS (Cardiovascular Health Study).

Methods

Data, analytical methods, and study materials will not be made available to other researchers for the purpose of reproducing the results or replicating the procedure. The authors are not authorized to share CHS data.

Study Design and Participants

CHS is a prospective cohort study supported by the National Heart, Lung, and Blood Institute to identify risk factors for and consequences of cardiovascular diseases in older adults. Participants were randomly selected from Medicare eligibility lists from 4 US communities: Sacramento County, CA; Washington County, MD; Forsyth County, NC; and Allegheny County, PA. Eligibility criteria were broad and included age ≥65 years and being noninstitutionalized, expected to remain in their current community for >3 years, and not under active hospice or cancer treatment. Among all eligible participants contacted, 57% agreed to participate. A total of 5888 men and women were initially recruited, including 5201 in 1989-1990 and an additional 687 black subjects to increase diversity in 1992-1993. Trained personnel performed annual study clinic examinations to assess participants' demographic characteristics, medical history, hospitalizations, health status, and lifestyle through standardized protocols.[46-49] Each center's institutional review committee approved the study protocols, and all participants provided written informed consent. For this analysis, we excluded 977 participants with prevalent HF at baseline and 662 participants without available blood for fatty acid measurements, resulting in 4249 participants in the current analysis.

Blood Sample Collection and Fatty Acid Analysis

Plasma phospholipid fatty acid concentrations were serially measured in all CHS participants with available stored blood samples in 1992-1993 (n=3693; 70% of living participants), 1998-1999 (n=2472; 62%), and 2005-2006 (n=902; 47%) included in the present analysis (Figure S1). Individual plasma phospholipid fatty acids were measured as a percentage of total phospholipid fatty acids analyzed by the Fred Hutchinson Cancer Research Center Biomarkers Laboratory. Blood samples were collected after a 12-hour fast, processed, and stored at −80°C. Total lipids were extracted from plasma,[50] and phospholipids were separated by using 1-dimensional thin-layer chromatography. Using Lepage's methods,[51] transesterified fatty acid methyl esters were prepared and then analyzed by using gas chromatography (initially 160°C for 16 minutes, ramped to 3.0°C/min to 240°C, and held for 15 minutes; Agilent [Santa Clara, CA] 5890 Gas Chromatograph Flame Ionization Detector and a Supelco fused silica capillary column SP-2560 [100 m×0.25 mm, 0.2 μm; Sigma-Aldrich, St. Louis, MO]). Identification, precision, and accuracy were tested by using model mixtures of known transesterified fatty acid methyl esters and an established in-house control pool, with identification confirmed by gas chromatography-mass spectrometry at the US Department of Agriculture lipid laboratory. Laboratory coefficients of variations were <3% for 16:0, 16:1n-7, 18:0, 18:1n-7, and 18:1n-9; and <8% for 14:0 and 16:1n-9.

Assessment of Incident Heart Failure and Ejection Fraction

Participants were followed by annual study clinic examinations with interim telephone contacts for 10 years and telephone every 6 months thereafter (1992-2015). History of HF before enrollment was identified by participant report and verified by medical record review.[48] During follow-up, incident HF was adjudicated by a centralized events committee using outpatient and inpatient medical records, diagnostic tests, clinical consultations, and interviews. Confirmation of definite HF required (1) diagnosis by a treating physician; (2) HF symptoms (shortness of breath, fatigue, orthopnea, and paroxysmal nocturnal dyspnea) plus signs (edema, rales, tachycardia, gallop rhythm, and displaced apical impulse) or supportive clinical findings on echocardiography, contrast ventriculography, or chest radiography; and (3) medical therapy for HF, defined as diuretics plus either digitalis or a vasodilator (angiotensin-converting enzyme inhibitors, hydralazine, and long-acting nitrates).[52]

Approximately 60% of participants who developed incident HF had data on left ventricular ejection fraction (EF) measurement at the time of HF diagnosis based on medical record review, derived from echocardiography, cardiac catheterization, multigated acquisition scanning, or other modalities. For the current study, we classified EF ≤40% as HF with reduced EF (HFrEF; n=295), EF 41% to 49% as HF with midrange EF (HFmrEF; n=82), and EF ≥50% as HF with preserved EF (HFpEF; n=402).

Assessment of Other Covariates

At the same study visits as the fatty acid measurements at baseline (1992-1993), 6 years (1998-1999), and 13 years (2005-2006), information on demographics, anthropometrics, blood pressure, medical history, lifestyle, and other risk factors was collected using standardized interviews, physical examinations, and laboratory testing.[46] Weight, height, and waist circumference were measured by using standardized methods with body mass index (BMI) (in kg/m^2) calculated as weight divided by height squared. Physical activity was assessed by using a modified Minnesota Leisure-Time Activities questionnaire.[53] Dietary intake was assessed in 1989-1990 using a 99-item validated food frequency questionnaire (National Cancer Institute)[54] and in 1995-1996 using a validated 131-item food frequency questionnaire.[55] Blood lipids were measured by using standardized methods,[50,51] and CRP (C-reactive protein) was measured by using a high-sensitivity enzyme-linked immunosorbent assay.[56]

Statistical Analyses

Unadjusted Spearman correlations (spearman command in Stata; Statacorp, College Station, TX) were used to evaluate intercorrelations between fatty acids in the DNL pathway at each time point and of repeated within-individual measures of each fatty acid over the 3 time points.

Cox proportional hazards models (stcox command in Stata) with time-varying exposures and covariates were used to estimate hazard ratios (HRs) associated with higher levels of each fatty acid in the DNL pathway, with time from fatty acid measurement at risk until HF, death, or the latest adjudicated date of follow-up in 2015. The Cox proportional hazards assumption was tested using Schoenfeld residuals,[57] indicating no evidence of violation for any of the fatty acids. Proportional hazards appeared violated for 3 covariates: sex, study site, and physical activity. We conducted sensitivity analyses utilizing joint risk-set stratification by categories of these covariates, demonstrating that the results derived from the stratified models were very similar to those from the nonstratified models (data not shown).

To evaluate habitual fatty acid levels over time, we used time-varying weighted cumulative averages: levels in 1992-1993 were related to risk of HF from 1992-1993 to 1998-1999; the average of levels in 1992-1993 and 1998-1999 to risk of HF from 1998-1999 to 2005-2006; and the average of levels in 1992-1993, 1998-1999, and 2005-2006, to risk of HF from 2005-2006 to 2015, with 50% weight assigned to the most recent measurement. For participants with missing fatty acid levels during follow-up (26% in 1998-1999, 48% in 2005-2006), prior measurements were carried forward. Compared with participants with no missing measures, those with missing fatty acid measurements were less likely to report excellent/very good health status and more likely to have drug-treated hypertension (Table S1). Fatty acids were modeled continuously according to the interquintile range, defined as the difference between the medians of the first and fifth quintiles. This continuous difference was selected for intuitive comparison to our additional nonlinear categorical (indicator) analyses in which we compared each quintile of fatty acid levels to the first quintile as the reference category, with quintile cutpoints based on study values in 1992-1993. Potential nonlinear associations were also explored semiparametrically using restricted cubic splines.

To evaluate changes in fatty acid levels over time, we calculated the mean percentage change in fatty acids for all participants with at least 2 fatty acid measurements (n=2032) (Figure S1). At each time period, the percentage change was calculated as the difference between the recent fatty acid measurement and the prior measurement, divided by the prior measurement and multiplied by 100. The percentage changes in fatty acid levels from 1992-1993 to 1998-1999 were related to risk of HF between 1998-1999 and 2005-2006; and the averaged percentage changes over the time periods from 1992-1993 to 1998-1999 and from 1998-1999 to 2005-2006 to risk of HF between 2005-2006 and 2015. Percentage changes in fatty acid levels were evaluated continuously according to their interquintile range.

To minimize potential confounding, covariates were selected based on biological interest, risk factors for HF in older adults, or associations with exposures and outcomes in the current population. Baseline covariates included age, sex, race, education, family history of cardiovascular disease, total energy intake, and enrollment site, time-varying covariates included smoking status, alcohol intake, prevalent diabetes mellitus, prevalent coronary heart disease (CHD), physical activity, BMI, waist circumference, and self-reported health status; and dietary covariates included consumption of fruits, vegetables, processed meat, red meats, fiber, carbohydrate, glycemic load, and whole grains; and plasma phospholipid concentrations of the long-chain n-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid. We also assessed as potential confounders or mediators factors that could be in the causal pathway between DNL and HF, ie, factors known to be influenced by hepatic steatosis and related metabolic pathways including levels of HDL-cholesterol, triglycerides, CRP, systolic blood pressure, and use of lipid-lowering medication and hypertension medication. Covariate data were imputed using multiple demographic and risk variables using single imputation (impute command in Stata) at each time point (0.1% to 6.4% in 1992-1993, 3.9% to 6.2% in 1998-1999, 4.6% to 10.8% in 2005-2006 for most factors; 2.7% to 12.4% for dietary factors; up to 20.4% in 1998-1999 and 41% in 2005-2006 for anthropometrics and blood measurements). Results were also evaluated when participants with missing

covariates were excluded and found to be very similar (data not shown).

Potential effect modification of the main fatty acid-HF associations was explored for age, sex, BMI, waist circumference, prevalent diabetes mellitus, and prevalent CHD by assessing the significance of multiplicative interaction terms using a Wald test.

A strength of the CHS cohort is available data on incidence of HF subtypes (HFrEF, HFmrEF, HFpEF). Given smaller numbers of events and no prespecified hypotheses for these subtypes, we performed exploratory analyses to assess the associations of each fatty acid with these subtypes. Given that patients with HFmrEF have a similar clinical profile and prognosis to those with HFpEF,[58] we combined HFmrEF with HFpEF in the analyses for HFpEF.

Sensitivity analyses were conducted excluding people with prevalent CHD at baseline; excluding events within the first 2 years of follow-up to minimize reverse causation (preexisting subclinical disease leading to changes in levels of fatty acids). For the main fatty acid-HF associations, we utilized a 2α of 0.05. We did not adjust for multiple comparisons, given prespecified hypotheses for DNL fatty acids and our primary outcome (HF), but were cautious 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. For the exploratory analyses of effect modification, we utilized a Bonferroni-corrected 2-tailed α of $0.05/42=0.001$. All analyses were performed using Stata 14.2.

Results

Baseline Characteristics

At baseline, mean (SD) age was 75.6 (5.3) years, 60% of participants were female, and 15% were nonwhite (Table 1). Most (79%) reported good or better self-reported health, 42% were former smokers, and 10% were current smokers. Mean BMI (SD) was 26.7 (4.6) kg/m^2, 12% of participants had prevalent diabetes mellitus, 15.6% had prevalent CHD, and 45% had drug-treated hypertension.

Median concentrations of fatty acids in the DNL pathway ranged from 0.09% (16:1n-9) to 25.3% (16:0) of total phospholipid fatty acids. Baseline characteristics stratified by quintiles of 16:0 are shown in Table S2. Participants with higher 16:0 levels were more likely to be white, more educated, higher in income, and former or current smokers. In addition, factors associated with increased DNL such as greater alcohol use, waist circumference, triglyceride levels, CRP, and prevalent diabetes mellitus were higher in those with higher levels of 16:0. In contrast, 16:0 levels were not

Table 1. Baseline Characteristics of US Men and Women in the CHS

Variable*	Participants (n=4249)
Demographics	
Age, mean (SD), y	75.6 (5.3)
Female, n (%)	2533 (60)
Race	
White, n (%)	3609 (85)
Nonwhite, n (%)	640 (15)
Education, n (%)	
<High school	1105 (26)
High school	1215 (29)
Some college	982 (23)
College graduate	947 (22)
Income group, n (%)	
<$11 999	956 (23)
$12 000-$24 999	1517 (36)
$25 000-$49 999	1200 (28)
>$50 000	576 (14)
Enrollment site, n (%)	
Bowman Gray	1069 (25)
Davis	1153 (27)
Hopkins	914 (22)
Pittsburgh	1113 (26)
Lifestyle	
Self-reported health status, n (%)	
Excellent/very good	1704 (40)
Good	1645 (39)
Fair/poor	900 (21)
Smoking, n (%)	
Never smoked	2000 (47)
Former smoker	1806 (42)
Current smoker	443 (10)
Physical activity, kcal	1154 (1374)
Alcohol, drinks/wk	2.5 (6.3)
BMI, kg/m^2	26.7 (4.6)
Waist circumference, cm	97.2 (13.0)
Systolic blood pressure, mm Hg	136 (21)
Diastolic blood pressure, mm Hg	71 (11)
Biochemical	
HDL cholesterol, mg/dL	53.6 (14.5)
Triglycerides, mg/dL	143.7 (85.1)
C-reactive protein, mg/L	5.1 (9.2)
Medical history	

Continued

Table 1. Continued

Variable*	Participants (n=4249)
Family history of myocardial infarction or stroke, n (%)	1273 (30)
Prevalent diabetes mellitus, n (%)	501 (12)
Prevalent coronary heart disease, n (%)	663 (15.6)
Lipid-lowering medication, n (%)	292 (7)
Hypertension medication, n (%)	1906 (44.9)
Dietary habits	
Total fat, % of energy	30.8 (5.2)
Carbohydrate, % of energy	53.8 (6.6)
Protein, % of energy	18.1 (2.6)
Fruits, servings/d	2.2 (1.0)
Vegetables, servings/d	3.0 (1.4)
Whole grains, g/d	33.3 (20.8)
Glycemic load	139.6 (41.0)
Energy intake, kcal/d	2001 (631)
Fatty acid biomarkers (% total fatty acids)	
Palmitic acid (16:0), median (IQR)	25.3 (23.5, 27.5)
Stearic acid (18:0), median (IQR)	13.5 (12.1, 14.9)
Palmitoleic acid (16:1n-7), median (IQR)	0.44 (0.28, 0.73)
Oleic acid (18:1n-9), median (IQR)	7.43 (6.29, 8.92)
Myristic acid (14:0), median (IQR)	0.27 (0.19, 0.37)
7-Hexadecanoic acid (16:1n-9), median (IQR)	0.09 (0.07, 0.12)
Vaccenic acid (18:1n-7), median (IQR)	1.28 (1.06, 1.56)

BMI indicates body mass index; CHS, Cardiovascular Health Study; HDL, high-density lipoprotein; IQR, interquintile range, the difference between the midpoint of first and fifth quintiles.

*Values reported as mean (SD) for continuous variables, and frequency, percentage (%) for categorical variables, unless otherwise stated; 3693 participants entered the study at 1992-1993, 526 participants at 1998-1999, and 30 participants at 2005-2006.

associated with estimated dietary intakes of saturated fat, carbohydrate, protein, or total energy.

Spearman correlations among the fatty acids at each time point are shown in Table S3. Generally, modest positive intercorrelations were seen (range 0.14-0.57) except for 18:0, which was inversely correlated with the other fatty acids (range −0.14 to −0.40). Correlations for repeated within-individual measures of each fatty acid over the 13 years of serial measurements ranged from 0.37 to 0.66 (Table S4).

Risk of HF: Habitual Levels of Fatty Acids in the DNL Pathway

During 45 030 person years of follow-up (maximum 22.1 years), 1304 HF cases occurred. In multivariable models adjusted for demographic, medical, lifestyle, and other HF risk factors, higher habitual levels of 16:0 were positively associated with risk of incident HF when evaluated continuously (HR [95% CI] per interquintile range 1.17 [1.00-1.36]; *P*=0.049; Figure 2) and in indicator quintiles (*P*-trend=0.018; Figure S2). Higher habitual levels of 18:1n-9 were associated with a trend toward higher HF risk when evaluated continuously (1.13 [0.98-1.30]) and with higher risk when evaluated in indicator quintiles (*P*-trend=0.039). Other fatty acids in the DNL pathway were not significantly associated with HF. Further adjustment for multiple dietary factors including fruits, vegetables, processed meat, red meats, fiber, carbohydrate, glycemic load, and whole grains as well as potential mediators had little effect on results. For example, after adjustment for dietary covariates, the HR (95% CI) per interquintile range for 16:0 was 1.16 (1.00-1.36).

When we evaluated the potential for nonlinear associations using restricted cubic splines, a potential nonlinear association was identified for 18:1n-9 (*P*-nonlinearity=0.001) but not for the other fatty acids (Figure 3).

Risk of HF: Changes Over Time in Levels of Fatty Acids in DNL Pathway

When changes in fatty acid levels were assessed, changes in levels of 16:0, 16:1n-9, and 18:1n-7 were each positively associated with risk of HF (interquintile HRs [95% CI]=1.26 [1.03-1.55], 1.36 [1.13-1.62], and 1.43 [1.18-1.72], respectively) (Table 2). Changes in levels of 18:0, 16:1n-7, 18:1n-9, and 14:0 were not significantly associated with risk.

Exploratory and Sensitivity Analyses

When HF subtypes were explored, results for both habitual levels and changes in levels of fatty acids were generally consistent with the main findings (Figures S3 and S4). Strongest associations were seen for changes in 16:0 (interquintile HR [95% CI]=1.86 [1.23-2.82]) and 16:1n-7 (interquintile HR [95% CI]=1.48 [1.02-2.15]) in relation to HFrEF. When individuals with HFmrEF were excluded from the analyses for HFpEF, findings were unchanged (Table S5).

Findings were similar after exclusion of participants with prevalent CHD at baseline (n=663) (Figure S5). For example, the interquintile HRs for habitual levels of 16:0 were 1.20 (1.01-1.43). Exclusion of events in the first 2 years to minimize reverse causation due to preexisting subclinical disease partly attenuated the positive association of 16:0 with HF risk (interquintile HRs [95% CI]=1.10 [0.94-1.30]) (Table S6).

Significant heterogeneity was not identified in the associations between fatty acids in the DNL pathway and HF according to age, sex, BMI, waist circumference, prevalent

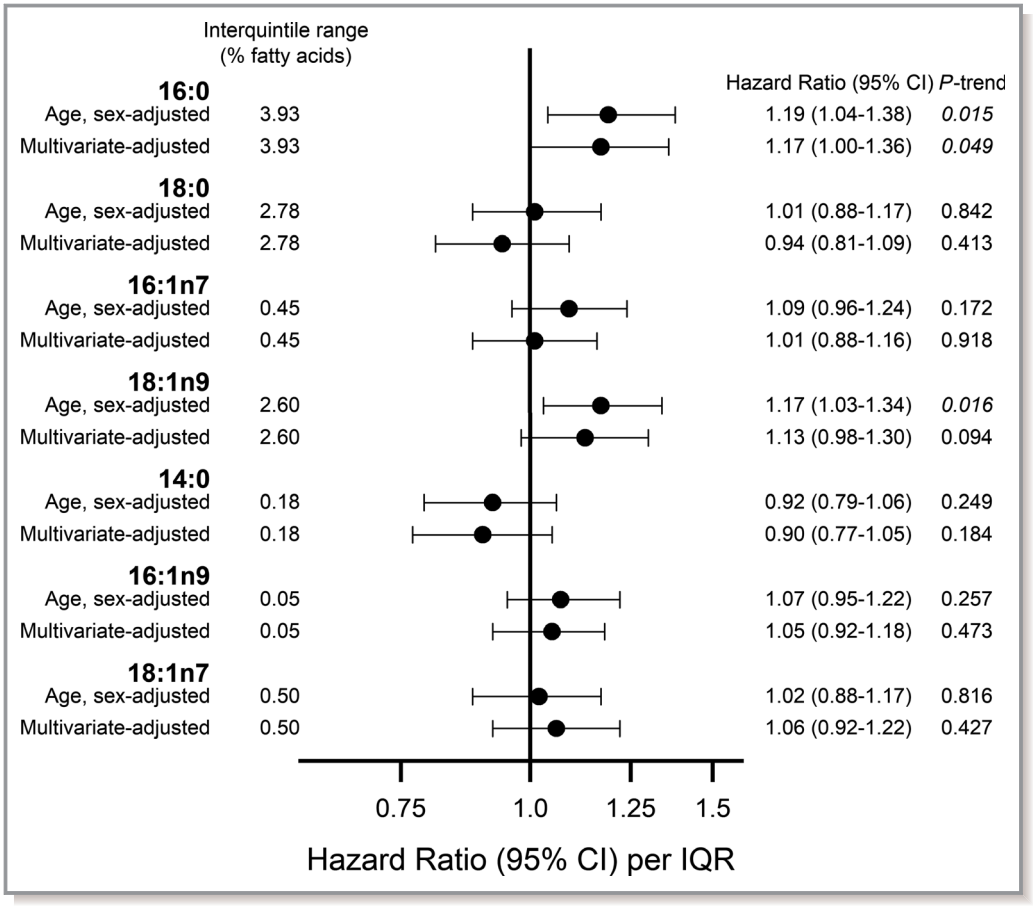

Figure 2. Hazard ratio of incident heart failure associated with habitual levels of plasma phospholipid fatty acids in the de novo lipogenesis pathway per interquintile range (IQR) among 4249 older men and women in the Cardiovascular Health Study. Fatty acids were measured at baseline, year 6, and year 13 with time-varying updating and covariates and followed up for 22 years. The IQR is the difference between the midpoint of the first and fifth quintiles. Multivariable adjustments include age (years), sex (male, female), race (white, nonwhite), enrollment (Bowman Gray, Davis, Hopkins, Pittsburgh), education (<high school, high school, some college, college graduate), income (<$11 999, $12 000 to $24 999, $25 000 to $49 999, >$50 000/y); physical activity (<500, 500-1000, 1000-1500, >1500 kcal/wk), waist circumference (cm), BMI (kg/m^2), self-reported health status (excellent/very good, good, fair/poor), alcohol intake (0, <1, 1-2.49, 2.5-7.49, 7.5-14.49, >14.5 drinks/week), smoking status (never, former, current), family history of myocardial infarction or stroke (yes/no), energy intake (kcal/d), plasma phospholipid n-3 fatty acids (% of total fatty acids), prevalent diabetes mellitus (yes/no), and prevalent CHD (yes/no). BMI indicates body mass index; CHD, coronary heart disease; IQR, interquintile range.

CHD at baseline, or prevalent diabetes mellitus at baseline (Bonferroni-corrected *P*-interaction>0.001 each) (Table S7).

Discussion

In this large, community-based prospective study among older US adults, both habitual levels and changes over time in plasma phospholipid 16:0 were positively associated with incident HF. Changes in levels of 16:1n-9 and 18:1n-7 were also positively associated with incident HF. Habitual levels and changes in levels of 14:0, 16:1n-7, 18:0, and 18:1n-9 were not significantly associated with HF risk. To our knowledge, this is the first study to prospectively assess serial biomarker levels of fatty acids in the DNL pathway, a crucial metabolic pathway for multiple HF risk factors, in relation to onset of HF.

Mechanistic studies support the biological plausibility of our findings for 16:0, which experimentally causes lipotoxicity, vascular calcification, and proinflammatory responses,[23,24,26,59] all of which could increase HF. In addition to direct effects, the observed association could reflect the role of 16:0 as a biomarker of DNL. Hepatic DNL contributes to intrahepatic fat accumulation and NAFLD. Growing evidence suggests that NAFLD is associated with

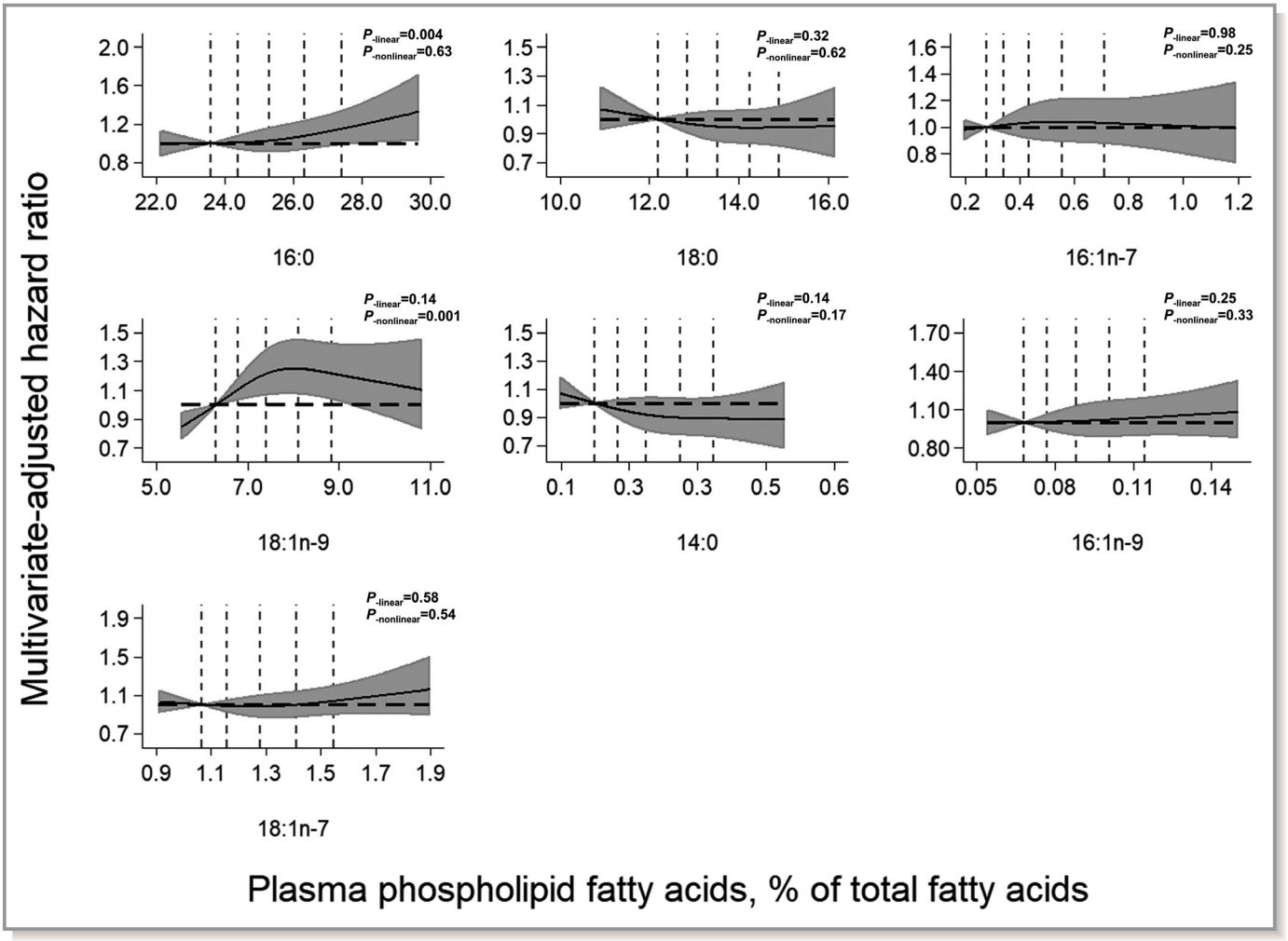

Figure 3. Multivariate-adjusted relationship of habitual levels of plasma phospholipid fatty acids in the de novo lipogenesis pathway with risk of heart failure, evaluated using restricted cubic splines. The solid lines and shaded areas represent the central risk estimates and 95% CIs, respectively, 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. *P* values for linear associations are presented. Evidence for nonlinearity 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. Significant nonlinearity was found for 18:1n-9 (*P*-nonlinearity=0.001), suggesting a possible threshold effect. Estimates were adjusted for potential confounders (see the footnote in Table 2).

impaired cardiac structure and function such as altered left ventricular geometry, lowered left ventricular filling pressure, and higher diastolic relaxation velocity, independent of risk factors for HF.[60-63] Although the biological mechanisms linking NAFLD and HF are not well established, NAFLD is associated with multiple risk factors for HF, including insulin resistance, visceral fat, atherogenic dyslipidemia, hypertension, and inflammation[13,64-66] that may collectively influence the development and progression of HF. Further studies are needed to explore biological mechanisms linking 16:0, directly and/or as a biomarker of DNL, to the development of HF.

Although 16:0 is the predominant saturated fatty acid in the diet, its direct dietary consumption has minor effects on circulating levels compared with DNL and endogenous metabolism. In cohort studies estimated dietary 16:0 consumption weakly correlates with circulating 16:0 (r=−0.02 to 0.09).[67-70] In a controlled crossover feeding trial, a wide variation of consumption of saturated fat from 11% to 30% of total energy did not change blood levels of saturated fatty acids including 16:0. In contrast, in isotope labeling studies, high-carbohydrate diets stimulate the synthesis of 16:0 via DNL.[71,72] This suggests that circulating 16:0 levels are largely driven by endogenous synthesis through DNL rather than direct dietary intake.

We found that long-term levels of changes in 16:0, 16:1n-9, and 18:1n-7 were positively associated with risk of HF. To our knowledge this is the first study having serial biomarker measures of fatty acids with many years of follow-up in a large cohort, allowing investigation of the relationship between

Table 2. Hazard Ratio of Incident Heart Failure Associated With Change in Serial Levels of Plasma Phospholipid Fatty Acids in the De Novo Lipogenesis Pathway Among 2032 Older Men and Women in the CHS

	Baseline Level, % of Total Fatty Acids	Mean Percentage Change Over Time in Fatty Acid Levels (IQR)*	Hazard Ratio (95% CI) Per IQR Increased Change in Level†	*P* Value
16:0	25.4 (24.3-26.3)	0.4 (−5.1, 6.2)	1.26 (1.03-1.55)	0.03
18:0	13.6 (12.8-14.3)	0.6 (−6.9, 8.2)	0.94 (0.76-1.15)	0.53
16:1n-7	0.5 (0.3-0.6)	−4.7 (−35.9, 26.9)	1.06 (0.87-1.28)	0.59
18:1n-9	7.5 (6.7-8.1)	2.2 (−12.7, 17.9)	1.13 (0.93-1.37)	0.23
14:0	0.28 (0.23-0.33)	4.9 (−23.1, 35.7)	1.11 (0.91-1.36)	0.30
16:1n-9	0.09 (0.08-0.10)	7.4 (−15.2, 31.7)	1.36 (1.13-1.62)	0.001
18:1n-7	1.3 (1.1-1.4)	4.0 (−9.9, 19.0)	1.43 (1.18-1.72)	<0.001

CHS indicates Cardiovascular Health Study; IQR, interquintile range.

Fatty acids were measured at baseline, year 6, and year 13. We looked at changes between baseline and year 6, and year 6 and year 13 with up to 16 years of follow-up.

*Values are the mean and interquintile range (midpoints of the first and fifth quintiles) of percentage changes in fatty acid levels, averaged over the time periods from 1992 to 1998 and from 1998 to 2005.

†Hazard ratios (95% CIs) associated with interquintile range (comparing the median of the first and fifth quintiles (ie, 10th to 90th percentiles). Multivariable adjustments include age (y), sex (male, female), race (white, nonwhite), 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/y); physical activity (<500, 500-1000, 1000-1500, >1500 kcal/week), waist circumference (cm), body mass index (kg/m^2), self-reported health status (excellent/very good, good, fair/poor), alcohol intake (0, <1, 1-2.49, 2.5-7.49, 7.5-14.49, >14.5 drinks/wk), smoking status (never, former, current), family history of myocardial infarction or stroke (yes/no), energy intake (kcal/d), plasma phospholipid n-3 fatty acids (% of total fatty acids), prevalent diabetes mellitus (yes/no), and prevalent coronary heart disease (yes/no).

changes over time in these fatty acids and incident HF. These novel findings suggest that changes in fatty acid levels may have greater biological relevance or sensitivity than habitual levels, due to their better capturing of changes in physiology and underlying risk over time. Given that no prior studies have evaluated serial measures to assess changes in these fatty acids with HF, our results support the need for additional research to investigate the role of changes (increase or decrease) in these fatty acids in the development of HF, using serial fatty acid measurements over time.

We did not observe significant associations of habitual levels or changes in levels of 14:0, 18:0, 16:1n-7, or 18:1n-9 with HF. In prior in vitro studies, 14:0 and 18:0 induce proinflammatory responses and apoptosis, whereas 16:1n-7 and 18:1n-9 have protective effects against insulin resistance.[30,33,34,73] Findings for these fatty acids for cardiovascular disease risk factors and events are limited or inconclusive in trials and observational studies.[8,17,41,74,75] Together with present findings, the potential effects of these fatty acids on HF remain unclear.

If additional investigation confirms adverse effects of 16:0, 16:1n-9, and 18:1n-7 or DNL on HF, DNL and its lipid metabolites could be targeted using different approaches to reduce risk of HF. Hepatic DNL is stimulated by the excess influx of dietary substrates (eg, free sugar, refined starch, alcohol)[71,72,76] and further exacerbated by any decrement in hepatic capacity to handle these substrates owing to metabolic dysfunction (eg, insulin resistance, NAFLD, metabolic syndrome).[77] Under such conditions, HF risk could be reduced by lowering intakes of sugar, refined starch, and alcohol; increasing physical activity to reduce visceral fat and build muscle mass, which in turn improves insulin sensitivity; and/or inhibiting key enzymes involved in fatty acid synthesis such as ATP citrate lyase, acetyl-CoA carboxylase, and fatty acid synthase as potential therapeutic targets.[16,78,79]

In exploratory analyses of HF subtypes we found positive associations between changes in 16:0 and 16:1n-7 and HFrEF. Although growing evidence suggests that NAFLD is associated with increased risk of left ventricular diastolic dysfunction,[63,80-82] neither habitual levels nor changes in fatty acids in the DNL pathway were associated with HFpEF. Our exploratory findings support the need to further investigate the biological mechanisms that may link changes in levels of 16:0 and 16:1n-7 with the pathogenesis of left ventricular systolic dysfunction. In sensitivity analyses we observed that the positive associations of habitual levels of 16:0 with HF were attenuated after exclusion of events in the first 2 years. These findings suggest the possibility of reverse causation; for example, undiagnosed subclinical HF may alter circulating levels of 16:0, or individuals with undiagnosed subclinical HF might have higher intakes of free sugar, refined starch, or alcohol. The partial attenuation of association could also be due to loss of power or chance. Our results support the need for further studies investigating the biological effects of 16:0 on HF.

Only 2 prior cohorts have evaluated associations of baseline plasma phospholipid fatty acids in the DNL pathway with risk of HF,[41-45] both in younger cohorts (mean±SD age 54.1±5.8 years in the ARIC [Atherosclerosis Risk in Communities] cohort and 58.7±8.0 years in the PHS [Physicians'

Health Study] cohort).[41,42] In ARIC, 16:0 positively associated with risk of HF (extreme quintile HR [95% CI]=2.16 [1.36-3.43]).[41] In PHS, 16:0 and 16:1n-7 positively, and 18:1n-7 inversely, associated with risk of HF (extreme quartile HR [95% CI]=1.49 [1.11-2.00] for 16:0; 1.58 [1.11-2.25] for 16:1n-7; and 0.23 [0.09-0.58] for 18:1n-7).[42,43,45] In both cohorts, 14:0, 18:0, and 18:1n-9 were not significantly associated with incident HF.[41,42,44] Differences between these 2 cohorts, as well as with our findings, may reflect variation in the study population ages (middle-aged adults in ARIC and PHS versus older adults in CHS), generalizability (US physicians in PHS versus community-based cohorts in ARIC and CHS), timing of exposure (single baseline measure in ARIC and PHS versus repeated serial measures in CHS), duration of follow-up (14.3 years for ARIC versus 22.1 years for CHS), or outcome assessment (self-reported HF in PHS versus medical records in ARIC and CHS). Neither of these prior observational studies evaluated the association of 16:1n-9 with risk of HF, highlighting the need for additional studies of this fatty acid. Our study builds on and greatly extends these previous findings in several respects. First, we assessed the association of DNL fatty acids with HF in older adults, the general population at highest risk. Second, we evaluated serial measures over 13 years to assess both habitual exposure and changes in these fatty acids and HF, using serially measured biomarkers over 13 years (versus baseline measurement only). Changes in fatty acid concentrations may have greater biological relevance or sensitivity than baseline levels due to better capturing of changes in physiology and underlying risk over time. Third, we explored the association of DNL fatty acids with HF subtypes (HFrEF, HFpEF), which has not been previously reported.

Our investigation has several strengths. We used serial measures of plasma phospholipid fatty acids in a large cohort with little loss to follow-up. Although some participants were missing follow-up fatty acid measures, the availability of serial measures in most of the participants is still a significant advance over all prior studies utilizing only a single measure at baseline. Circulating biomarkers provided objective measures of fatty acid concentrations compared with a single measurement of fatty acid or self-reported dietary data. A large number of events provided statistical power to detect relevant associations. Information on demographic characteristics, lifestyle habits, and risk factors of HF was prospectively collected using standardized methods, allowing multivariable adjustment to minimize confounding. HF outcomes were identified and confirmed by a comprehensive review and centralized adjudication process, which decreased the potential for missed or misclassified outcomes.

Potential limitations should be considered. Our study population included older adults, mostly white, and our findings may not be generalizable to younger populations or other races. As an observational study, residual confounding by unknown or unmeasured factors may be present. However, our results were robust to adjustment for multiple major risk factors for HF as well as socioeconomic status and dietary and other lifestyle habits. Information on ejection fraction was available for 60% of events, and findings for HF subtypes should be interpreted with caution. We did not adjust for multiple comparisons for the separate primary hypotheses. Circulating fatty acids in the DNL pathway may reflect not only DNL but also direct dietary consumption and metabolism. However, given that directly measured hepatic DNL through isotope labeling in a large cohort study is extremely expensive and impractical, these fatty acids have been shown to be reasonable biomarkers of DNL.[19]

In conclusion, both habitual levels and changes in 16:0 were positively associated with risk of HF in older adults. Changes in 16:1n-9 and 18:1n-7 were also positively associated with HF. Our findings support the need for experimental and interventional studies to elucidate the potential mechanistic link between habitual levels and changes in levels of these fatty acids as well as DNL and incidence of HF.

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SUPPLEMENTAL MATERIAL

Table S1. Baseline characteristics for participants by fatty acid measurements in the Cardiovascular Health Study.

Variable	Participants with ≥2 or more FA measurements* (n=2,032)	Participants with ≥1 FA measurement† (n=4,249)
Demographics		
Age, mean (SD), years	73.6 (4.4)	75.6 (5.3)
Female, n (%)	1,280 (63)	2,533 (60)
Race		
White, n (%)	1,759 (87)	3,609 (85)
Non-White, n (%)	273 (13)	640 (15)
Education, n (%)		
<High school	453 (22)	1,105 (26)
High school	577 (28)	1,215 (29)
Some college	522 (26)	982 (23)
College graduate	480 (24)	947 (22)
Income group, n (%)		
<$11,999	374 (18)	956 (23)
$12,000-$24,999	699 (34)	1,517 (36)
$25,000-$49,999	642 (32)	1,200 (28)
>$50,000	317 (16)	576 (14)
Enrollment site, n (%)		
Bowman Gray	534 (26)	1,069 (25)
Davis	597 (29)	1,153 (27)
Hopkins	448 (22)	914 (22)
Pittsburgh	453 (22)	1,113 (26)
Lifestyle		
Self-reported health status, n (%)		
Excellent/very good	985 (48)	1,704 (40)
Good	752 (37)	1,645 (39)
Fair/poor	295 (15)	900 (21)
Smoking, n (%)		
Never smoked	1,018 (50)	2,000 (47)
Former smoker	828 (41)	1,806 (42)
Current smoker	186 (9)	443 (10)
Physical activity, kcal	1,283 (1,444)	1,154 (1,374)
Alcohol, drinks/wk	2.7 (6.3)	2.5 (6.3)
BMI, kg/m2	26.9 (4.4)	26.7 (4.6)
Waist circumference, cm	97.2 (12.8)	97.2 (13.0)
Systolic blood pressure, mmHg	135 (20)	136 (21)
Diastolic blood pressure, mmHg	71 (11)	71 (11)
Biochemical		
HDL cholesterol, mg/dL	54.1 (14.3)	53.6 (14.5)
Triglycerides, mg/dL	143.1 (84.4)	143.7 (85.1)
C-reactive protein, mg/L	3.1 (5.7)	5.1 (9.2)
Medical history		
Family history of myocardial infarction or stroke, n (%)	616 (30)	1,273 (30)
Prevalent diabetes, n (%)	200 (10)	501 (12)
Prevalent coronary heart disease, n (%)	238 (12)	663 (15.6)
Lipid lowering medication, n (%)	106 (5)	292 (7)
Hypertension medication, n (%)	814 (40.1)	1,906 (44.9)
Dietary habits		
Total fat, % of energy	30.4 (5.0)	30.8 (5.2)

Variable	Participants with ≥2 or more FA measurements* (n=2,032)	Participants with ≥1 FA measurement† (n=4,249)
Carbohydrate, % of energy	54.3 (6.3)	53.8 (6.6)
Protein, % of energy	17.9 (2.4)	18.1 (2.6)
Fruits, servings/d	2.2 (1.0)	2.2 (1.0)
Vegetables, servings/d	3.1 (1.4)	3.0 (1.4)
Whole grains, grams/d	32.5 (18.8)	33.3 (20.8)
Glycemic load	139.0 (38.0)	139.6 (41.0)
Energy intake, kcal/d	1,962 (598)	2001 (631)
Fatty acid biomarkers (% total fatty acids), median (IQR)		
Palmitic acid (16:0)	25.2 (23.5, 27.4)	25.3 (23.5, 27.5)
Stearic acid (18:0)	13.5 (12.2, 14.9)	13.5 (12.1, 14.9)
Palmitoleic acid (16:1n-7)	0.45 (0.29, 0.73)	0.44 (0.28, 0.73)
Oleic acid (18:1n-9)	7.34 (6.26, 8.77)	7.43 (6.29, 8.92)
Myristic acid (14:0)	0.27 (0.20, 0.37)	0.27 (0.19, 0.37)
7-hexadecanoic acid (16:1n-9)	0.09 (0.07, 0.11)	0.09 (0.07, 0.12)
Vaccenic acid (18:1n-7)	1.26 (1.05, 1.52)	1.28 (1.06, 1.56)

* Participants who had 2 or more fatty acid measurements (n=2,032) were included in the analysis assessing the relation of changes in fatty acid levels with heart failure.

† Participants who had at least 1 fatty acid measurement (n=4,249) were included in the analysis assessing the relation of long-term levels of fatty acids with heart failure.

IQR= interquintile range, the difference between the midpoint of 1st and 5th quintile

Table S2. Baseline characteristics of US men and women in the Cardiovascular Health Study by quintiles of palmitic acid (16:0).

	Palmitic acid (16:0)				
	Q1 (n=834)	Q2 (n=852)	Q3 (n=843)	Q4 (n=855)	Q5 (n=865)
Fatty acids, % of total fatty acids	23.5 (23.0, 23.9)	24.5 (24.3, 24.7)	25.3 (25.1, 25.5)	26.1 (25.9, 26.3)	27.5 (27.0, 28.2)
Demographics					
Age, years	75.2 ± 5.3	75.4 ± 5.4	75.6 ± 5.5	76.2 ± 5.4	75.4 ± 5.2
Female, %	68.7	59.4	56.0	53.9	60.2
Race					
White, %	77.9	80.6	85.5	88.9	91.4
Education, %					
<High school	30.6	30.0	26.1	23.8	19.6
High school	32.0	30.6	24.9	26.5	29.0
Some college	21.5	18.2	26.2	24.6	24.8
College graduate	15.9	21.1	22.8	25.0	26.7
Income group, %					
<$11,999	29.6	25.3	21.2	23.0	17.9
$12,000-$24,999	35.1	37.5	35.5	34.1	32.1
$25,000-$49,999	24.7	24.5	28.8	26.9	31.6
>$50,000	10.6	12.7	14.5	16.0	18.4
Enrollment site, %					
Bowman Gray	33.1	27.9	25.6	20.1	19.3
Davis	23.0	24.1	26.5	28.8	33.2
Hopkins	22.4	23.4	21.0	22.1	18.7
Pittsburgh	21.5	24.6	26.9	29.0	28.8
Lifestyle					
Self-reported health status, %					
Excellent/very good/good	79.6	79.9	83.6	83.0	83.2
Fair/poor	20.4	20.1	16.4	17.0	16.8
Smoking, %					
Never smoked	54.4	48.4	46.1	45.6	41.0

	Palmitic acid (16:0)				
	Q1 (n=834)	Q2 (n=852)	Q3 (n=843)	Q4 (n=855)	Q5 (n=865)
Former smoker	35.7	41.3	43.1	45.1	47.1
Current smoker	9.8	10.3	10.8	9.2	11.9
Physical activity, kcals	1018 ± 1173	1099 ± 1323	1180 ± 1415	1267 ± 1496	1198 ± 1420
Alcohol, drinks/wk	0.8 ± 2.8	1.7 ± 4.5	1.8 ± 5.3	3.0 ± 6.2	5.3 ± 9.7
BMI, kg/m2	25.9 ± 4.3	26.5 ± 4.6	27.2 ± 4.7	27.0 ± 4.9	27.0 ± 4.4
Waist circumference, cm	94.6 ± 12.5	96.7 ± 13.0	97.9 ± 13.0	98.4 ± 13.3	98.3 ± 12.9
Systolic blood pressure, mmHg	137 ± 22	135 ± 21	137 ± 21	135 ± 21	137 ± 21
Diastolic blood pressure, mmHg	71.7 ± 10.8	71.1 ± 10.9	71.4 ± 11.2	70.1 ± 11.4	70.4 ± 11.3
Biochemical					
HDL cholesterol, mg/dL	55.4 ± 14.1	52.6 ± 13.3	52.5 ± 14.1	52.0 ± 13.9	55.6 ± 16.5
Triglycerides, mg/dL	125 ± 62	131 ± 69	143 ± 84	149 ± 92	170 ± 104
C-reactive protein, mg/L	3.0 ± 4.5	3.4 ± 6.5	3.5 ± 7.4	3.5 ± 4.9	4.1 ± 7.3
Medical history					
Family history of myocardial infarction or stroke, %	32.5	30.9	29.4	27.8	29.2
Prevalent diabetes, %	9.5	11.7	13.9	13.0	18.0
Prevalent coronary heart disease, %	16.2	15.3	15.4	16.3	14.9
Lipid lowering medication, %	9.1	5.3	7.1	5.3	7.6
Hypertension medication, %	45.0	44.0	42.6	44.7	48.0
Dietary habits					
Total fat, % of energy	31.1 ± 4.9	31.2 ± 5.0	30.9 ± 5.1	30.8 ± 5.1	30.2 ± 5.6
Saturated fat, % of energy	9.9 ± 2.1	10.1 ± 2.2	10.2 ± 2.1	10.2 ± 2.2	10.1 ± 2.3
Carbohydrate, % of energy	54.2 ± 6.3	53.8 ± 6.3	53.9 ± 6.4	53.7 ± 6.4	53.5 ± 7.5
Protein, % of energy	17.8 ± 2.4	17.8 ± 2.5	18.1 ± 2.6	18.1 ± 2.6	18.5 ± 2.7

	Palmitic acid (16:0)				
	Q1 (n=834)	Q2 (n=852)	Q3 (n=843)	Q4 (n=855)	Q5 (n=865)
Fruits, servings/d	2.2 ± 1.0	2.2 ± 1.1	2.3 ± 1.1	2.2 ± 1.0	2.2 ± 1.0
Vegetables, servings/d	3.0 ± 1.4	3.0 ± 1.4	3.0 ± 1.5	2.9 ± 1.3	3.0 ± 1.4
Whole grains, grams/d	32.1 ± 19.5	32.8 ± 20.1	34.0 ± 22.5	32.5 ± 20.0	35.1 ± 21.7
Glycemic load	141 ± 37	141 ± 44	142 ± 43	139 ± 41	136 ± 41
Energy intake, kcal/d	2006 ± 693	2007 ± 665	2028 ± 623	1995 ± 606	1972 ± 564

Table S3. Unadjusted Spearman correlation coefficients for plasma phospholipid fatty acids in the de novo lipogenesis pathway in men and women in Cardiovascular Health Study*.

	Correlations in the same year						
	16:0	**18:0**	**16:1n-7**	**18:1n-9**	**14:0**	**16:1n-9**	**18:1n-7**
1992-1993 (n=3,693)							
18:0	-0.45						
16:1n-7	0.56	-0.22					
18:1n-9	0.31	-0.28	0.56				
14:0	0.40	-0.16	0.60	0.29			
16:1n-9	0.05*	-0.15	0.40	0.41	0.20		
18:1n-7	0.00*	-0.38	0.17	0.23	-0.20	0.34	
1998-1999 (n=2,472)							
18:0	-0.51						
16:1n-7	0.53	-0.28					
18:1n-9	0.26	-0.29	0.52				
14:0	0.40	-0.14	0.57	0.26			
16:1n-9	0.03*	-0.15	0.43	0.46	0.14		
18:1n-7	-0.00*	-0.36	0.22	0.27	-0.18	0.42	
2005-2006 (n=902)							
18:0	-0.57						
16:1n-7	0.47	-0.24					
18:1n-9	0.33	-0.32	0.54				
14:0	0.32	-0.06*	0.57	0.25			
16:1n-9	0.08	-0.16	0.50	0.53	0.17		
18:1n-7	0.06*	-0.40	0.27	0.25	-0.22	0.44	

* All correlations were significant ($P < 0.05$) except for the correlations of 16:0 with 18:1n-7 at all time points, of 16:0 with 16:1n-9 in 1992-93 and 1998-99, and of 14:0 with 18:0 in 2005-2006 ($P>0.05$).

Table S4. Correlations among repeated within-individual measures of each fatty acid over three time points*

		16:0	
	1992 (n=3,693)	**1998 (n=2,472)**	**2005 (n=902)**
16:0			
1992	1.00		
1998	0.62	1.00	
2005	0.53	0.53	1.00
		18:0	
18:0			
1992	1.00		
1998	0.64	1.00	
2005	0.54	0.54	1.00
		16:1n-7	
16:1n-7			
1992	1.00		
1998	0.66	1.00	
2005	0.54	0.64	1.00
		18:1n-9	
18:1n-9			
1992	1.00		
1998	0.50	1.00	
2005	0.40	0.44	1.00
		14:0	
14:0			
1992	1.00		
1998	0.48	1.00	
2005	0.37	0.50	1.00
		16:1n-9	
16:1n-9			
1992	1.00		
1998	0.49	1.00	
2005	0.38	0.48	1.00
		18:1n-7	
18:1n-7			
1992	1.00		
1998	0.63	1.00	
2005	0.49	0.59	1.00

*Values were estimated using Spearman correlations. All correlations are significant (P<0.0001).

Table S5. Exploratory analyses for the associations between habitual levels of phospholipid fatty acids in the de novo lipogenesis and the risk of heart failure with preserved ejection fraction after excluding patients with heart failure with mid-range ejection fraction*

	Habitual levels of fatty acids in the de novo lipogenesis pathway	Changes in fatty acids in the de novo lipogenesis pathway
	HR per IQR (95% CI)	HR per IQR (95% CI)
Cases/total	402/4,249	231/2,032
16:0	1.03 (0.77-1.36)	0.98 (0.70-1.37)
18:0	0.95 (0.73-1.25)	1.09 (0.78-1.51)
16:1n-7	1.00 (0.77-1.29)	0.91 (0.65-1.27)
18:1n-9	1.22 (0.93-1.59)	0.93 (0.67-1.29)
14:0	0.80 (0.60-1.08)	1.06 (0.75-1.49)
16:1n-9	1.07 (0.85-1.33)	1.28 (0.93-1.74)
18:1n-7	1.17 (0.90-1.51)	1.33 (0.97-1.83)

*Individuals with heart failure with mid-range ejection fraction (HFmrEF: 41-49 % ejection fraction; n=82) were excluded from the analysis. 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/y); physical activity (<500, 500-1000, 1000-1500, >1500 kcal/wk), waist circumference (cm), BMI (kg/m^2), self-reported health status (excellent/very good, good, fair/poor), alcohol intake (0, <1, 1-2.49, 2.5-7.49, 7.5-14.49, >14.5 drinks/wk), smoking status (never, former, current), family history of myocardial infarction or stroke (yes/no), energy intake (kcal/d), plasma phospholipid omega-3 fatty acids (% of total fatty acids), prevalent diabetes (yes/no), and prevalent coronary heart disease (yes/no). HR = hazard ratio; IQR = interquintile range; CI = confidence interval.

Table S6. Sensitivity analyses for the associations between habitual levels of phospholipid fatty acids in the de novo lipogenesis and risk of heart failure after excluding cases (n=123) in the first 2 years of follow-up*

	Full follow-up	Excluding cases in the first 2 years
	HR per IQR (95% CI)	HR per IQR (95% CI)
16:0	1.17 (1.00-1.36)	1.10 (0.94-1.30)
18:0	0.94 (0.81-1.09)	0.95 (0.81-1.11)
16:1n-7	1.10 (0.88-1.16)	1.00 (0.86-1.15)
18:1n-9	1.13 (0.98-1.30)	1.09 (0.94-1.27)
14:0	0.90 (0.77-1.05)	0.81 (0.69-0.96)
16:1n-9	1.05 (0.92-1.18)	0.95 (0.82-1.11)
18:1n-7	1.06 (0.92-1.22)	1.08 (0.93-1.25)

*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/y); physical activity (<500, 500-1000, 1000-1500, >1500 kcal/wk), waist circumference (cm), BMI (kg/m^2), self-reported health status (excellent/very good, good, fair/poor), alcohol intake (0, <1, 1-2.49, 2.5-7.49, 7.5-14.49, >14.5 drinks/wk), smoking status (never, former, current), family history of myocardial infarction or stroke (yes/no), energy intake (kcal/d), plasma phospholipid omega-3 fatty acids (% of total fatty acids), prevalent diabetes (yes/no), and prevalent coronary heart disease (yes/no). HR = hazard ratio; IQR = interquintile range; CI = confidence interval.

Table S7. Phospholipid fatty acids in the de novo lipogenesis pathway and the risk of heart failure in the Cardiovascular Health Study: analysis of potential interaction by age, sex, body mass index, waist circumference, prevalent coronary heart disease and prevalent diabetes with respective stratified analyses with Bonferroni correction (significance<0.001)*

	16:0		18:0		16:1n-7		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	1.27 (1.02, 1.59)	0.99	0.98 (0.79, 1.23)	0.67	1.23 (1.02, 1.47)	0.60	1.31 (1.06, 1.60)	0.05
>75 years	1.06 (0.85, 1.31)		0.95 (0.77, 1.18)		0.82 (0.66, 1.01)		0.98 (0.80, 1.20)	
Sex								
Female	1.28 (0.99, 1.66)	0.42	0.95 (0.74, 1.22)	0.69	1.10 (0.89, 1.36)	0.25	1.14 (0.93, 1.40)	0.96
Male	1.12 (0.92, 1.36)		0.93 (0.77, 1.13)		0.97 (0.81, 1.17)		1.13 (0.93, 1.38)	
BMI								
Normal	0.99 (0.76, 1.28)	0.96	1.23 (0.95, 1.59)	0.79	1.00 (0.79, 1.27)	0.59	1.10 (0.87, 1.38)	0.91
Overweight	1.30 (1.02, 1.65)		0.75 (0.59, 0.95)		0.97 (0.78, 1.22)		1.06 (0.84, 1.33)	
Obese	1.43 (1.01, 2.01)		1.00 (0.71, 1.40)		1.09 (0.82, 1.46)		1.24 (0.88, 1.73)	
Waist circumference								
≤97 cm	1.00 (0.80, 1.26)	0.25	1.11 (0.89, 1.38)	0.90	0.98 (0.79, 1.22)	0.50	1.10 (0.90, 1.35)	0.68
>97 cm	1.37 (1.10, 1.70)		0.80 (0.65, 0.99)		1.05 (0.88, 1.27)		1.19 (0.97, 1.46)	
Prevalent coronary heart disease								
Yes	1.34 (0.87, 2.09)	0.50	0.87 (0.56, 1.34)	0.80	1.34 (0.89, 2.02)	0.07	1.67 (1.13, 2.46)	0.11
No	1.13 (0.96, 1.34)		0.96 (0.81, 1.13)		0.97 (0.84, 1.13)		1.07 (0.92, 1.25)	
Prevalent diabetes								
Yes	1.22 (0.85, 1.75)	0.94	0.72 (0.51, 1.01)	0.55	0.85 (0.62, 1.16)	0.32	0.74 (0.52, 1.05)	0.01
No	1.16 (0.97, 1.37)		0.99 (0.83, 1.17)		1.05 (0.90, 1.23)		1.23 (1.05, 1.44)	

***** 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/y); physical activity (<500, 500-1000, 1000-1500, >1500 kcal/wk), waist circumference (cm), BMI (kg/m^2), self-reported health status (excellent/very good, good, fair/poor), alcohol intake (0, <1, 1-2.49, 2.5-7.49, 7.5-14.49, >14.5 drinks/wk), smoking status (never, former, current), family history of myocardial infarction or stroke (yes/no), energy intake (kcal/d), plasma phospholipid omega-3 fatty acids (% of total fatty acids), prevalent diabetes (yes/no), and prevalent coronary heart disease (yes/no). CI = confidence interval.

† P-value obtained from continuous interaction term for age, BMI, waist circumference, and categorical interaction term for sex (females as reference), prevalent coronary heart disease (no as reference) and prevalent diabetes (no as reference).

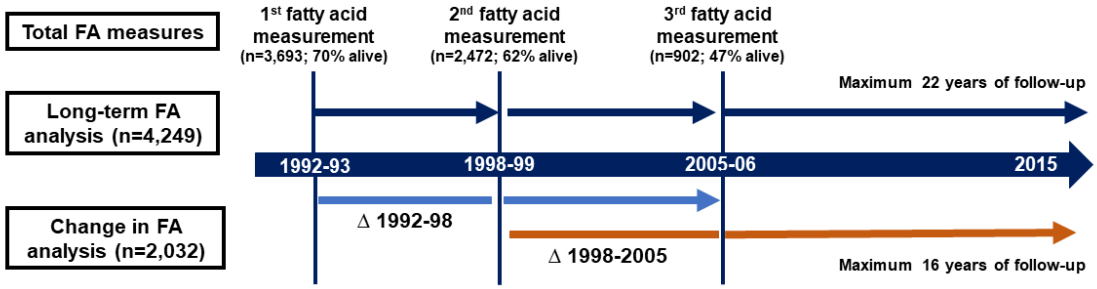
Table S7. Phospholipid fatty acids in the de novo lipogenesis pathway and the risk of heart failure in the Cardiovascular Health Study: analysis of potential interaction by age, sex, body mass index, waist circumference, prevalent coronary heart disease and prevalent diabetes with respective stratified analyses with Bonferroni correction (significance<0.001)* (continued)*

	14:0		16:1n-9		18:1n-7	
	HR (95% CI)	*P*-value†	HR (95% CI)	*P*-value†	HR (95% CI)	*P*-value†
Age						
≤75 years	1.02 (0.80, 1.29)	0.54	1.12 (0.98, 1.29)	0.02	0.99 (0.79, 1.24)	0.59
>75 years	0.82 (0.67, 1.02)		0.90 (0.75, 1.08)		1.04 (0.87, 1.26)	
Sex						
Female	0.94 (0.74, 1.20)	0.47	1.12 (0.92, 1.36)	0.69	1.02 (0.82, 1.27)	0.33
Male	0.89 (0.72, 1.10)		1.01 (0.86, 1.20)		1.08 (0.89, 1.31)	
BMI						
Normal	1.08 (0.84, 1.40)	0.08	1.07 (0.87, 1.31)	0.50	0.96 (0.76, 1.21)	0.94
Overweight	0.88 (0.68, 1.12)		1.14 (0.91, 1.43)		1.12 (0.88, 1.41)	
Obese	0.76 (0.53, 1.10)		0.87 (0.64, 1.18)		1.08 (0.78, 1.51)	
Waist circumference						
≤97 cm	1.03 (0.82, 1.29)	0.004	1.09 (0.91, 1.32)	0.44	0.91 (0.74, 1.12)	0.57
>97 cm	0.83 (0.66, 1.03)		1.02 (0.86, 1.21)		1.24 (1.01, 1.52)	
Prevalent coronary heart disease						
Yes	0.99 (0.63, 1.55)	0.27	1.33 (0.87, 2.03)	0.21	1.12 (0.73, 1.74)	0.98
No	0.88 (0.74, 1.04)		1.03 (0.90, 1.18)		1.06 (0.91, 1.24)	
Prevalent diabetes						
Yes	0.80 (0.52, 1.22)	0.62	0.75 (0.53, 1.07)	0.04	0.90 (0.62, 1.30)	0.15
No	0.91 (0.77, 1.09)		1.13 (0.98, 1.30)		1.10 (0.95, 1.29)	

* 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/y); physical activity (<500, 500-1000, 1000-1500, >1500 kcal/wk), waist circumference (cm), BMI (kg/m^2), self-reported health status (excellent/very good, good, fair/poor), alcohol intake (0, <1, 1-2.49, 2.5-7.49, 7.5-14.49, >14.5 drinks/wk), smoking status (never, former, current), family history of myocardial infarction or stroke (yes/no), energy intake (kcal/d), plasma phospholipid omega-3 fatty acids (% of total fatty acids), prevalent diabetes (yes/no), and prevalent coronary heart disease (yes/no). CI = confidence interval.

† P-value obtained from continuous interaction term for age, BMI, waist circumference, and categorical interaction term for sex (females as reference), prevalent coronary heart disease (no as reference) and prevalent diabetes (no as reference).

Figure S1. Participants included in the analysis evaluating the association of long-term and changes in levels of fatty acids in the de novo lipogenesis pathway with risk of heart failure.

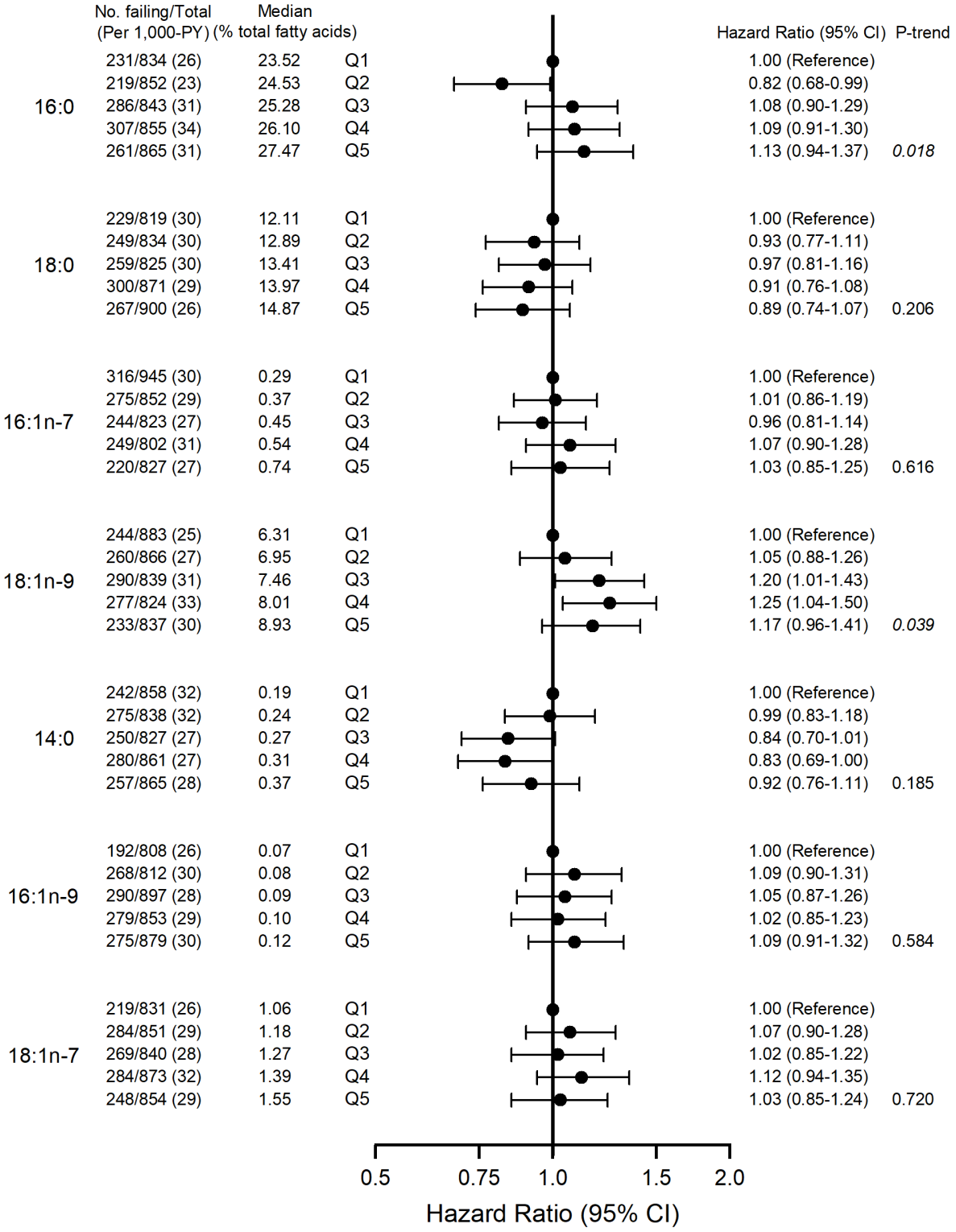


Long-term level of FA = 50% current measurement + 50% prior measurement(s)

$$\text{Change in level of FA} = \frac{\text{Recent measurement – prior measurement}}{\text{Prior measurement}} \times 100$$

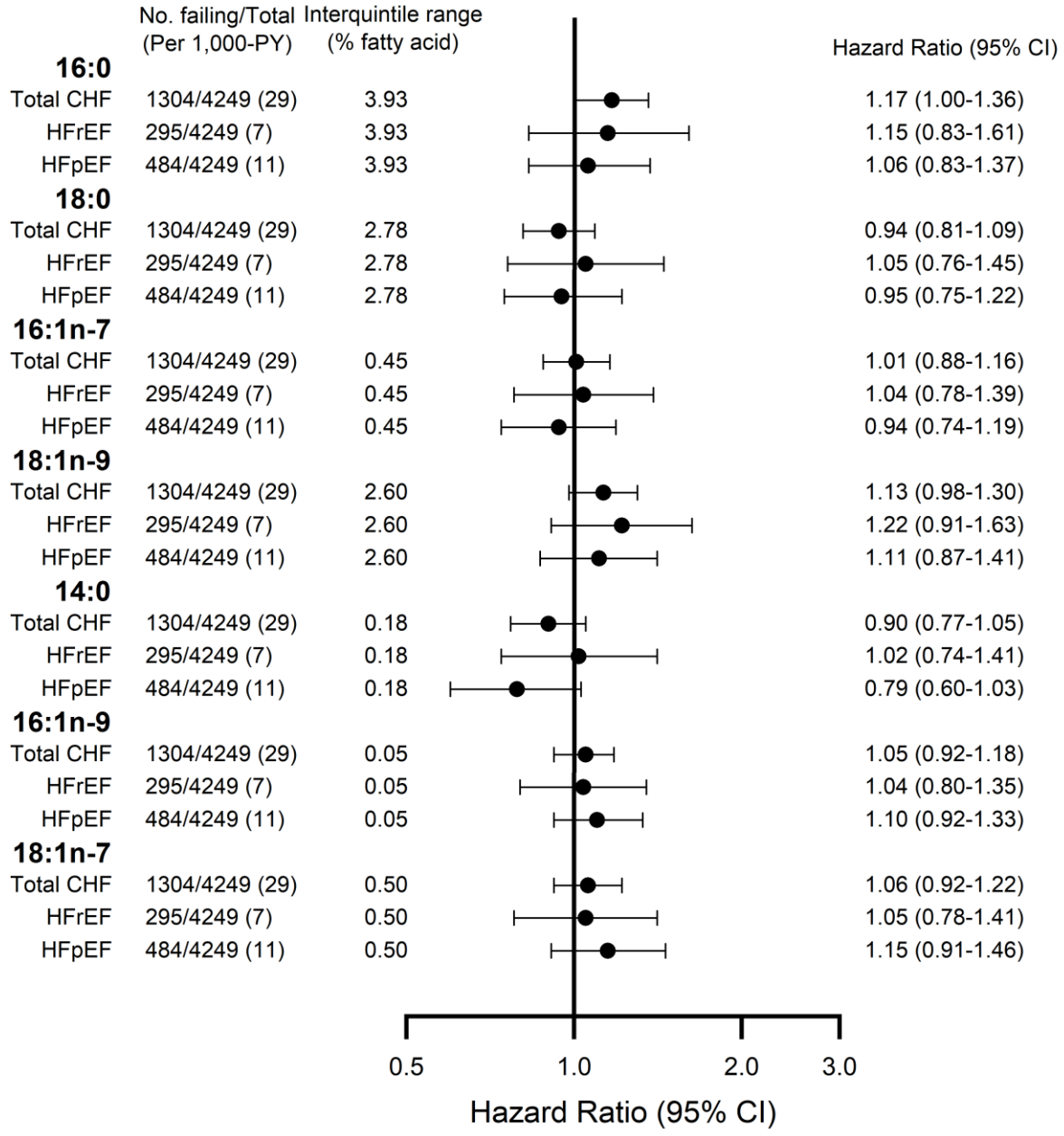
In the long-term fatty acid analysis, among 4,249 participants, 3,693 participants entered the study in 1992-93, 526 participants in 1998-99, and 30 participants in 2005-06. To evaluate long-term levels over time, we used time-varying weighted cumulative averages: levels in 1992-93 were related to risk of heart failure from 1992-93 to 1998-99; the average of levels in 1992-93 and 1998-99, to risk of heart failure from 1998-99 to 2005-06, and the average of levels in 1992-93, 1998-99, and 2005-06, to risk of heart failure from 2005-06 to 2015, with 50% weight assigned to the most recent measurement. In the change in fatty acid analysis, 2,032 participants who had ≥2 fatty acid measures were included. At each time period, the percent change was calculated as the difference between the recent fatty acid measurement and the prior measurement, divided by the prior measurement and multiplied by 100. The percent changes in fatty acid levels from 1992-93 to 1998-99 were related to risk of heart failure between 1998-99 and 2005-06; and the averaged percent changes over the time periods from 1992-93 to 1998-99 and from 1998-99 to 2005-06, to risk of heart failure between 2005-06 and 2015. FA, fatty acid

Figure S2. Relative risk of heart failure (n=1,304) associated with habitual levels of plasma phospholipid fatty acids in the de novo lipogenesis pathway per quintile among 4,249 older men and women in the Cardiovascular Health Study.



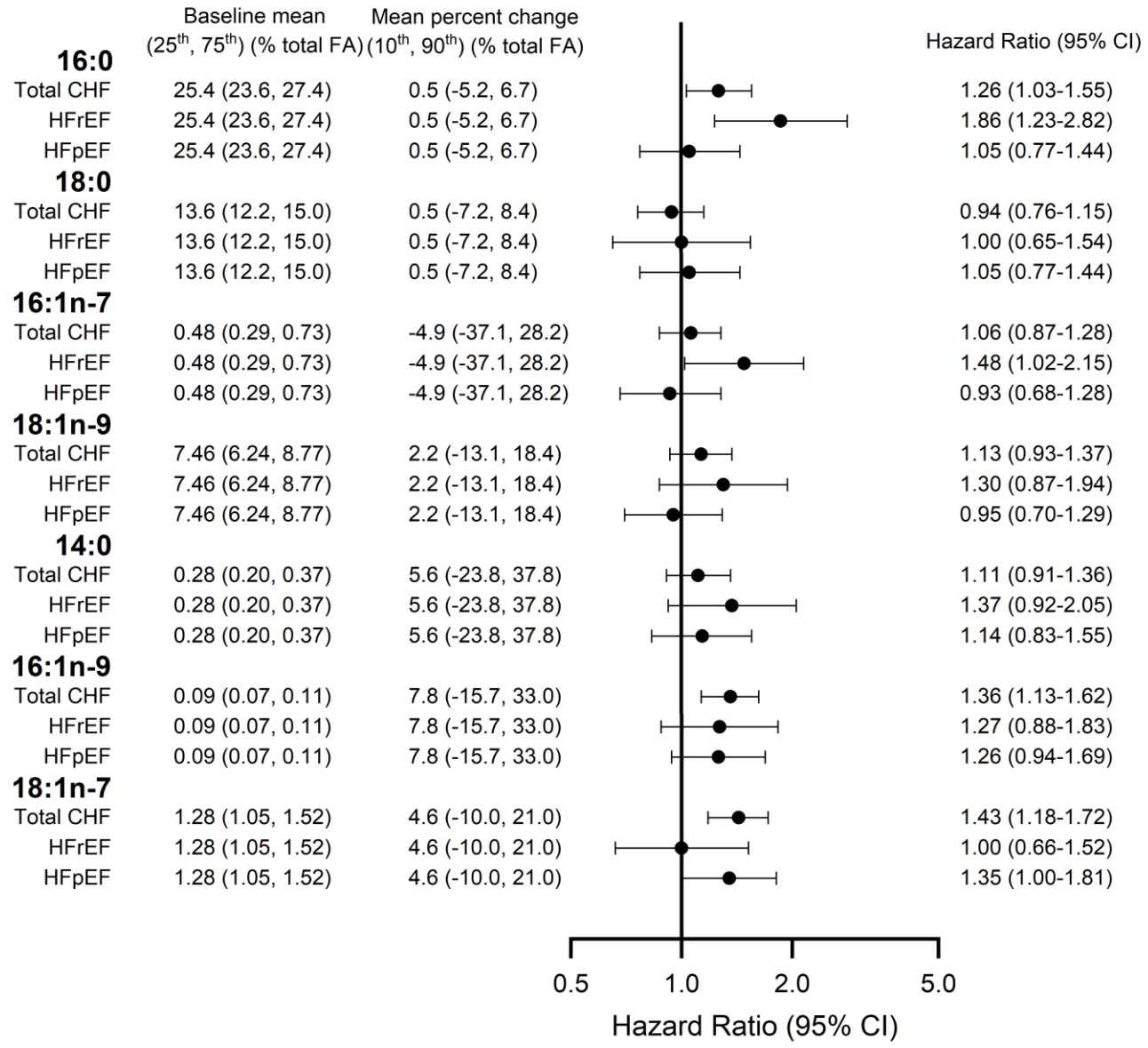
Fatty acids were measured at baseline, year 6, and year 13 with time varying updating and covariates, and follow-up for 22 years. P-trend was calculated by assessing quintiles as continuous variables after assigning participants the median value in each quintile. Multivariable adjustments include age (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/y); physical activity (<500, 500-1000, 1000-1500, >1500 kcal/wk), waist circumference (cm), BMI (kg/m^2), self-reported health status (excellent/very good, good, fair/poor), alcohol intake (0, <1, 1-2.49, 2.5-7.49, 7.5-14.49, >14.5 drinks/wk), smoking status (never, former, current), family history of myocardial infarction or stroke (yes/no), energy intake (kcal/d), plasma phospholipid omega-3 fatty acids (% of total fatty acids), prevalent diabetes (yes/no), and prevalent coronary heart disease (yes/no). CI = confidence interval; PY, person-years.

Figure S3. Relative risk of total heart failure, heart failure with reduced ejection fraction (HFrEF), and heart failure with preserved ejection fraction (HFpEF) associated with habitual levels of plasma phospholipid fatty acids in the de novo lipogenesis pathway per interquintile range (IQR) among 4,249 older men and women in the Cardiovascular Health Study.



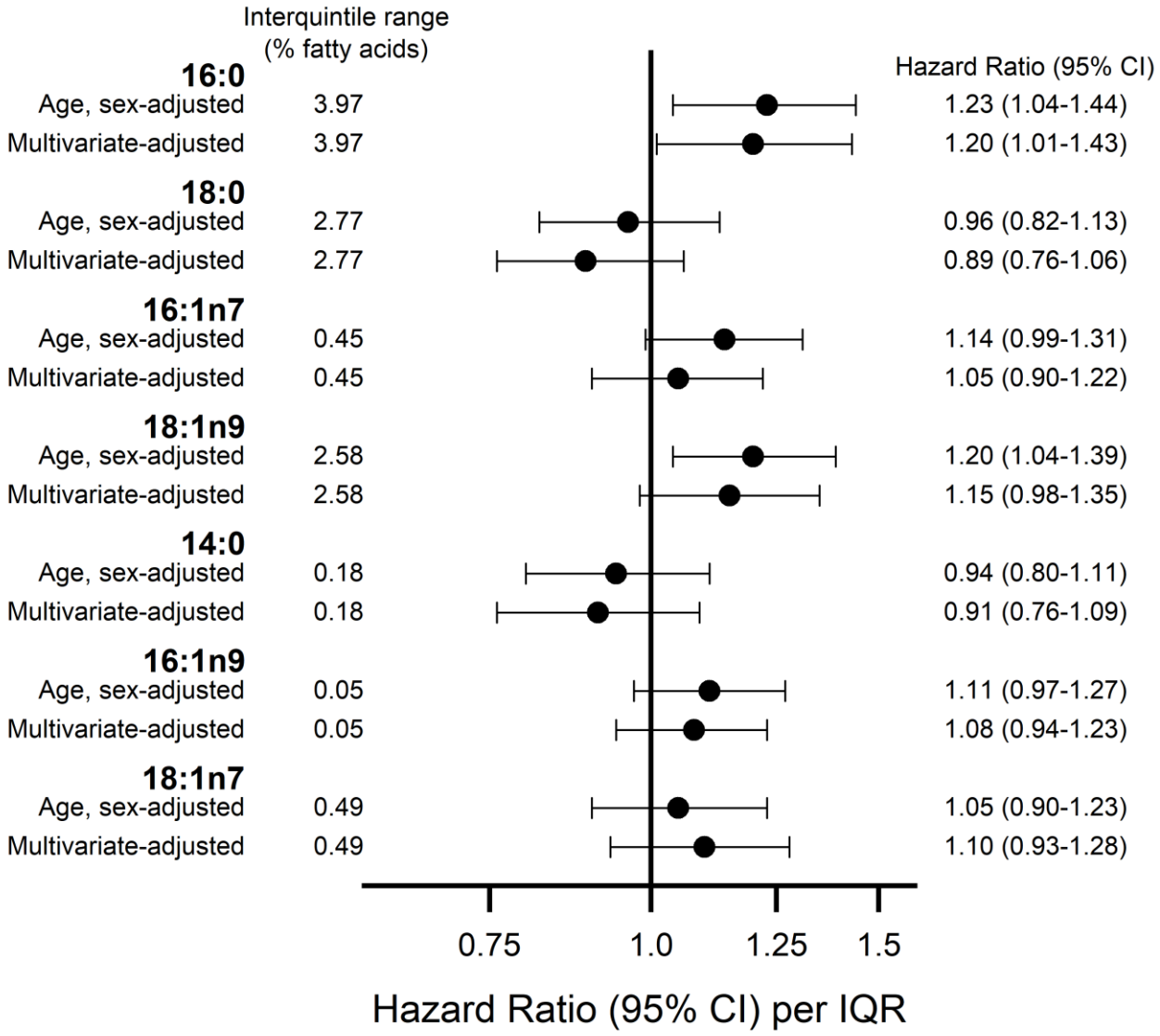
Fatty acids were measured at baseline, year 6, and year 13 with time varying updating and covariates, and follow-up for 22 years. The IQR is the difference between the midpoint of the 1st and 5th quintile. Multivariable adjustments include age (years), sex (male, female), race (white, non-white), enrollment 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/y); physical activity (<500, 500-1000, 1000-1500, >1500 kcal/wk), waist circumference (cm), BMI (kg/m^2), self-reported health status (excellent/very good, good, fair/poor), alcohol intake (0, <1, 1-2.49, 2.5-7.49, 7.5-14.49, >14.5 drinks/wk), smoking status (never, former, current), family history of myocardial infarction or stroke (yes/no), energy intake (kcal/d), plasma phospholipid omega-3 fatty acids (% of total fatty acids), prevalent diabetes (yes/no), and prevalent coronary heart disease (yes/no). CI = confidence interval.

Figure S4. Relative risk of total heart failure (n=659), heart failure with reduced ejection fraction (HFrEF, n=137), and heart failure with preserved ejection fraction (HFpEF, n=266) associated with change in serial levels of plasma phospholipid fatty acids in the de novo lipogenesis pathway per interquintile range (IQR) among 2,032 older men and women in the Cardiovascular Health Study.



Fatty acids were measured at baseline, year 6, and year 13. We looked at changes between baseline and year 6, and year 6 and year 13 with up to 22 years of follow-up. The IQR is the difference between the midpoint of the 1st and 5th 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/y); physical activity (<500, 500-1000, 1000-1500, >1500 kcal/wk), waist circumference (cm), BMI (kg/m^2), self-reported health status (excellent/very good, good, fair/poor), alcohol intake (0, <1, 1-2.49, 2.5-7.49, 7.5-14.49, >14.5 drinks/wk), smoking status (never, former, current), family history of myocardial infarction or stroke (yes/no), energy intake (kcal/d), plasma phospholipid omega-3 fatty acids (% of total fatty acids), prevalent diabetes (yes/no), and prevalent coronary heart disease (yes/no). CHF= congestive heart failure; FA= fatty acid; CI = confidence interval

Figure S5. Relative risk of heart failure associated with habitual levels of plasma phospholipid fatty acids in the de novo lipogenesis pathway per interquintile range (IQR) among 3,586 older men and women without prevalent coronary heart disease in the Cardiovascular Health Study.



Fatty acids were measured at baseline, year 6, and year 13 with time varying updating and covariates, and follow-up for 22 years. The IQR is the difference between the midpoint of the 1st and the 5th 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/y); physical activity (<500, 500-1000, 1000-1500, >1500 kcal/wk), waist circumference (cm), BMI (kg/m^2), self-reported health status (excellent/very good, good, fair/poor), alcohol intake (0, <1, 1-2.49, 2.5-7.49, 7.5-14.49, >14.5 drinks/wk), smoking status (never, former, current), family history of myocardial infarction or stroke (yes/no), energy intake (kcal/d), plasma phospholipid omega-3 fatty acids (% of total fatty acids), prevalent diabetes (yes/no), and prevalent coronary heart disease (yes/no). CI = confidence interval, IQR = interquintile range.