ORIGINAL RESEARCH

Circulating Very Long-Chain Saturated Fatty Acids and Heart Failure: The Cardiovascular Health Study

Rozenn N. Lemaitre, PhD, MPH; Barbara McKnight, PhD; Nona Sotoodehnia, MD, MPH; Amanda M. Fretts, PhD; Waqas T. Qureshi, MD, MS; Xiaoling Song, PhD; Irena B. King, PhD; Colleen M. Sitlani, PhD; David S. Siscovick, MD, MPH; Bruce M. Psaty, MD, PhD; Dariush Mozaffarian, MD, DrPH

***Background*—**Circulating very-long-chain saturated fatty acids (VLSFAs) are integrated biomarkers of diet and metabolism that may point to new risk pathways and potential targets for heart failure (HF) prevention. The associations of VLSFA to HF in humans are not known.

***Methods and Results*—**Using a cohort study design, we studied the associations of serially measured plasma phospholipid VLSFA with incident HF in the Cardiovascular Health Study. We investigated the associations of time-varying levels of the 3 major circulating VLSFAs, lignoceric acid (24:0), behenic acid (22:0), and arachidic acid (20:0), with the risk of incident HF using Cox regression. During 45030 person-years among 4249 participants, we identified 1304 cases of incident HF, including 489 with preserved and 310 with reduced ejection fraction. Adjusting for major HF risk factors and other circulating fatty acids, higher levels of each VLSFAs were associated with lower risk of incident HF (P trend≤0.0007 each). The hazard ratio comparing the highest quintile to the lowest quintile was 0.67 (95% confidence interval, 0.55–0.81) for 24:0, 0.72 (95% confidence interval, 0.60–0.87) for 22:0 and 0.72 (95% confidence interval, 0.59–0.88) for 20:0. The associations were similar in subgroups defined by sex, age, body mass index, coronary heart disease, and diabetes mellitus. Among those with ejection fraction data, the associations appeared similar for those with preserved and with reduced ejection fraction.

***Conclusions*—**Higher levels of circulating VLSFAs are associated with lower risk of incident HF in older adults. These novel associations should prompt further research on the role of VLSFA in HF, including relevant new risk pathways.

***Clinical Trial Registration*—**URL: https://www.clinicaltrials.gov. Unique identifier: NCT00005133. **(*J Am Heart Assoc*. 2018;7: e010019. DOI: 10.1161/JAHA.118.010019.)**

Key Words: epidemiology • fatty acid • heart failure

Heart failure (HF) is a disabling clinical syndrome with high rates of hospitalization and mortality.[1–3] With growing prevalence, health costs, and utilization, HF is a major public health issue. Prevalence of HF in the United States was estimated at 6.5 million in 2012, and ≈1 million new cases are diagnosed annually.[1] In addition, incidence of HF increases dramatically with advancing age, making HF a particular burden in the elderly.[1,4] Elucidation of novel dietary and metabolic determinants of HF may lead to improved pathophysiological understanding and potential new treatments.

Circulating very-long-chain saturated fatty acids (VLSFAs), saturated fatty acids (FAs) with 20 carbons or more, are integrated biomarkers of diet and metabolism. VLSFAs are

From the Departments of Medicine, Cardiovascular Health Research Unit (R.N.L., N.S., C.M.S., B.M.P.), Biostatistics (B.M.), Epidemiology (A.M.F., B.M.P.), and Health Services (B.M.P.), University of Washington, Seattle, WA; School of Medicine, Wake Forest University, Winston Salem, NC (W.T.Q.); Public Health Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, WA (X.S.); Department of Internal Medicine, University of New Mexico, Albuquerque, NM (I.B.K.); Institute for Urban Health, New York Academy of Medicine, New York, NY (D.S.S.); Kaiser Permanente Washington Health Research Institute, Seattle, WA (B.M.P.); Friedman School of Nutrition Science & Policy, Tufts University, Boston, MA (D.M.).

Accompanying Tables S1 and S2 and Figure S1 through S3 are available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.118.010019

Correspondence to: Rozenn N. Lemaitre, PhD, MPH, University of Washington, 1730 Minor Ave, Ste 1360, Seattle, WA 98101. E-mails: rozenl@uw.edu; rozenl@u.washington.edu

Received August 10, 2018; accepted September 14, 2018.

Clinical Perspective

What Is New?

- Higher levels of circulating very-long-chain saturated fatty acids are prospectively associated with lower risk of new heart failure in a large cohort of 4249 older adults.
- The very-long-chain saturated fatty acids were associated with lower risk of both heart failure with preserved ejection fraction and heart failure with reduced ejection fraction.
- The associations were independent of major risk factors for heart failure and similar in subgroups defined by sex, baseline age, body mass index, diabetes mellitus, and coronary heart disease.

What Are the Clinical Implications?

- Circulating very-long-chain saturated fatty acids are new biomarkers of heart failure risk.
- Future studies of dietary and metabolic determinants of the fatty acids are needed.
- If the associations prove to be causal, raising levels of very-long-chain saturated fatty acids may support the prevention of heart failure.

major components of ceramides and sphingomyelins, lipids made of a sphingosine backbone with 1 acylated fatty acid.[5] Ceramides in particular are known for their role in apoptosis,[6] and apoptosis appears crucial to the pathophysiology of HF.[7,8] Whereas ceramides with the long-chain saturated FA, palmitic acid (16:0 [16 carbons, 0 double bond]), promote apoptosis, ceramides with a VLSFA, especially 24:0, appear to counteract these effects and protect against apoptosis.[9] In particular, ceramide with 16:0 creates channels in the outer membrane of mitochondria, resulting in membrane permeabilization and apoptosis, but ceramide with 24:0, which is able to span both outer and inner membranes, is thought to destabilize the 16:0-ceramide–mediated channels, thereby preventing mitochondrial membrane permeabilization and apoptosis.[10] In addition, higher levels of circulating VLSFAs have been reported to be associated with lower risk of type 2 diabetes mellitus,[11,12] atrial fibrillation,[13] and coronary disease,[14] all important risk factors for HF. However, the relationship between VLSFA and HF has not been evaluated.

Given that VLSFA may influence the risk of HF through multiple mechanisms, we hypothesized that higher circulating VLSFA levels would be associated with lower risk of HF. We tested this hypothesis in the CHS (Cardiovascular Health Study), a well-characterized, prospective, population-based cohort study of cardiovascular disease risk in older adults with 3 serial biomarker measurements of plasma phospholipid FAs: at baseline and 6 and 13 years of follow-up. Using these unique data, we investigated the prospective association of the 3 major circulating VLSFAs, arachidic acid (20:0), behenic acid (22:0), and lignoceric acid (24:0), with risk of incident HF.

Methods

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

Study Population

CHS[15] participants were recruited from 4 US communities (Forsyth County, North Carolina; Sacramento County, California; Washington County, Maryland; and Allegheny County, Pennsylvania) from a random sample generated from the Health Care Financing Administration files. Among eligible adults who were contacted, 57% agreed to participate. The cohort consists of 5201 noninstitutionalized men and women, aged ≥65 years, recruited in 1989–1990, plus an additional 687 predominantly black participants recruited in 1992–1993. Each center's institutional review board approved the study, and all participants provided informed written consent to participate in the study.

Plasma phospholipid FAs were measured in blood drawn in 1992–1993, the baseline of our investigation, and again at 6 (1998–1999) and 13 years (2005–2006) of follow-up. We included 4249 CHS participants with at least 1 available FA measurement and free of prevalent HF at the time of their first FA measurement.

Plasma Phospholipid FAs

Serial FA measurements were obtained on 3693 participants at baseline (70.1% of living participants at this visit), 2472 participants at 6 years (62.2%), and 902 participants at 13 years (47.4%). At each study visit, blood was drawn after 12-hour fasting, and plasma specimens were stored at −70°C. Plasma lipids were subsequently extracted by the method of Folch[16] and phospholipids separated from other lipids by thin-layer chromatography. FA methyl esters were prepared by direct transesterification of the phospholipid fraction[17] and separated by gas chromatography using a fused-silica 100-m capillary column, as previously described.[18] FAs were expressed as weight % of total FAs. Laboratory personnel were blinded to the case status of the samples. Quality control included the use of a control sample run in parallel with each batch of study samples through the whole protocol. Interassay coefficients of variation for VLSFA measurements across the study period were ≤3.5%.

Ascertainment of HF

Participants were followed by annual clinic examinations with interim 6-month phone contacts until 1999, twice-yearly

telephone contacts thereafter, and another clinic visit in 2005–2006. Incident HF was adjudicated by a centralized committee using information from out- and inpatient medical records, diagnostic tests and consultations, and interviews. Confirmation of definite HF required each of 3 criteria: (1) diagnosis of HF by a treating physician; (2) either HF symptoms (shortness of breath, fatigue, orthopnea, or 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, or long-acting nitrates).[19] Whenever possible, HF was subtyped into HF with preserved ejection fraction (HFpEF) and HF with reduced ejection fraction (HFrEF) on the basis of findings from echocardiography and cardiac catheterization reports.

Other Risk Factors

Information on a wide range of covariates was obtained during study visits, including medical history, lifestyle, and clinical risk factors.[15] Information on age, sex, ethnicity, education, physical activity, and smoking status was based on self-report. Usual walking habits included average pace (gait speed) and distance walked. Weight, waist circumference, and height were measured using standardized protocols. Plasma lipids and glucose were assessed on fasting blood samples using enzymatic methods.[15] Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Diabetes mellitus was defined by a fasting glucose level of ≥7 mmol/L (126 mg/dL) or use of insulin or oral hypoglycemic agents.

Statistical Analyses

Levels of VLSFA were evaluated in quintiles as indicator variables, based on the distribution at study baseline, with the significance of trends across categories evaluated by entering the categorical variable as an ordinal variable. VLSFA levels were also evaluated linearly across the interquintile range, corresponding to the difference between the midpoint of the top and bottom quintiles (90th and 10th percentiles). Cox proportional hazards regression was used to estimate the hazard ratio (HR) of incident HF, with left entry at the first FA measurement and time at risk until first HF diagnosis, death, or latest date of adjudicated follow-up in 2015. Multivariable models included prespecified adjustments for age, sex, race, enrollment site, prevalent coronary heart disease, atrial fibrillation, diabetes mellitus, fasting glucose, treated hypertension, systolic blood pressure, BMI, waist circumference, smoking status, physical activity, and levels of 2 phospholipid FAs (20:5n3 [eicosapentaenoic acid] and 24:1n9 [nervonic acid]) previously associated with HF in this cohort.[18,20] Levels of VLSFA and model covariates were evaluated as time-varying variables with updating at the time of each new FA measurement. We also evaluated atrial fibrillation and coronary heart disease as time dependent, updating anytime during the follow-up; results from these models (not shown) were similar to results of primary models updating only at time of VLSFA update.

To further examine the shape of the VLSFA associations with HF risk, we used cubic spline models. For all but 1 of the VLSFAs, results from the fit of the spline model were consistent with the quintile-based results presented here (Figures S1 through S3). For the FA, 24:0, spline results indicated an elevation in risk at the highest levels, whereas in the quintile analysis, the highest quintile appeared to carry the lowest risk. To explore these discrepancies, we examined the distribution of 24:0 levels in the highest quintile and its association with risk. When we plotted a lowess smooth of Martingale residuals from a Cox model containing only the adjustment variables for subjects in the highest quintile of 24:0 (not shown), we observed that it was only the influence of sparse data for the highest values of 24:0 in the top quintile (2.0% and above) that suggested higher risk. For this reason, we retained the quintile analysis.

To examine the influence of lack of proportional hazards observed with some of the adjustment variables, we conducted sensitivity analyses incorporating true, risk-set stratification on categories defined by these combined variables. Results obtained with the stratified models (not shown) were similar to the results from the primary analyses presented here.

In secondary analysis, we compared the HRs for the associations of VLSFAs with HF with preserved ejection fraction (HFpEF) to the HRs for the associations of VLSFAs with HF with reduced ejection fraction (HFrEF), using the Lunn and McNeil approach.[21]

For each VLSFA, we explored potential effect modification by age, sex, BMI, diabetes mellitus, and prevalent coronary heart disease in models that included a linear term for the VLSFAs and a multiplicative term between the VLSFAs and the effect modifier. We used 0.05 for significance of 2-sided statistical tests. All analyses were conducted in Stata/SE software (version 14.2; StataCorp LP, College Station, TX).

Results

At baseline, participant mean age (SD) was 75.6 (5.3) years, and 40.4% were male. Mean (10th, 90th percentiles) VLSFA levels were 0.50% (0.40, 0.60) for 20:0, 1.66% (1.27, 2.07) for 22:0, and 1.39% (1.04, 1.76) for 24:0. For each VLSFA, within-individual correlations between the repeated measures were

Table 1. Levels and Correlations of Serial Measurements of Plasma Phospholipid VLSFAs in the Cardiovascular Health Study

	24:0	22:0	20:0
Levels at entry, % of total FAs			
Mean±SD	1.39±0.29	1.66±0.32	0.50±0.08
Median (range), IQR	1.37 (0.52–3.42), 0.72	1.65 (0.16–3.49), 0.80	0.50 (0.26–0.82), 0.21
Correlations of serial measurements			
Baseline and year 6	0.59	0.62	0.72
Baseline and year 13	0.48	0.48	0.60
Year 6 and year 13	0.56	0.57	0.67
Intercorrelations between VLSFAs at baseline			
24:0	1.0		
22:0	0.89	1.0	
20:0	0.47	0.64	1.0

20:0 indicates arachidic acid; 22:0, behenic acid; 24:0, lignoceric acid; IQR, interquintile range, defined as the difference between the midpoint of the top and bottom quintiles; VLSFA, very-long-chain saturated fatty acids.

≥0.48 (Table 1). Intercorrelations between baseline levels of the different VLSFAs were 0.64 (for 20:0 and 22:0), 0.47 (20:0 and 24:0), and 0.89 (22:0 and 24:0).

Table 2 shows participants characteristics across quintiles of 24:0. Participants in the highest quintile were, on average, younger, more likely to be male, less likely to be white, less

Table 2. Baseline Characteristics of 4249 Participants in the Cardiovascular Health Study, According to Quintiles of 24:0 Levels

	Q1	Q2	Q3	Q4	Q5
24:0, median (range), % of total FAs	1.05 (0.52–1.15)	1.24 (1.15–1.30)	1.37 (1.30–1.43)	1.52 (1.44–1.61)	1.76 (1.61–3.41)
Age, y	76.29±5.52	76.02±5.40	75.67±5.28	75.21±5.23	74.63±5.12
Sex, male (%)	31.89	37.62	41.30	45.62	45.72
Race, white (%)	89.93	88.10	85.56	85.66	75.50
Education, >high school, (%)	74.23	71.60	72.60	76.72	74.88
Current smoker, %	7.89	10.34	9.70	9.00	9.61
Diabetes mellitus, %	20.46	13.10	12.31	12.80	12.78
Coronary heart disease, %	20.69	21.27	19.17	21.33	15.94
Atrial fibrillation, %	9.94	7.57	6.15	5.92	4.92
Treated hypertension, %	58.63	51.92	45.92	45.85	43.85
Systolic blood pressure, Hg mm	138.34±22.11	136.38±21.67	136.35±20.32	134.26±19.93	135.20±21.42
Diastolic blood pressure, Hg mm	70.44±11.38	70.17±11.28	71.05±11.27	71.26±10.85	71.82±10.80
BMI, kg/m^2	27.25 ±5.03	26.75 ±4.84	26.55 ±4.57	26.43 ±4.39	26.39 ±4.31
Fasting glucose, mg/dL	113.10±37.48	106.32±29.27	105.91±30.86	108.10±40.07	106.67±33.83
Waist circumference, cm	98.88 ±14.30	97.33 ±13.40	97.09 ±12.50	96.44 ±12.17	96.14 ±12.68
LDL cholesterol, mg/dL	108.72±32.87	122.55±31.10	130.00±30.41	135.08±32.38	139.96±33.67
HDL cholesterol, mg/dL	52.29±15.97	53.48±14.72	53.41±13.97	53.10±13.59	54.60±13.63
Triglycerides, mg/dL	200.73±134.85	150.70±71.18	135.07±62.80	125.80±55.57	110.95±49.67
Blocks walked in previous week	30.66 ±54.03	41.69± 76.83	39.43 ±61.31	47.19±70.67	46.52±73.24

Q1, Q2, Q3, Q4, and Q5 are first, second, third, fourth, and fifth quintile, respectively. 24:0 indicates lignoceric acid; BMI, body mass index; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

Table 3. Association of Plasma Phospholipid Very-Long-Chain Saturated Fatty Acids With Incident Heart Failure in the Cardiovascular Health Study

	Quintile of Plasma Phospholipid Fatty Acid Levels					
	Q1	Q2	Q3	Q4	Q5	*P* Trend
24:0						
Cases/person-years	315/8675	297/9348	258/9064	245/9055	189/8888	
Incidence rate, ‰	3.63%	3.18%	2.85%	2.71%	2.13%	
Demographics adjusted*	REF	0.85 (0.72–1.00)	0.78 (0.66–0.92)	0.74 (0.62–0.87)	0.61 (0.51–0.74)	6.0×10^{-8}
Multivariable adjusted*	REF	0.91 (0.78–1.07)	0.91 (0.77–1.08)	0.86 (0.72–1.02)	0.73 (0.61–0.88)	0.001
Multivariable+FA adjusted*	REF	0.86 (0.74–1.02)	0.86 (0.72–1.01)	0.79 (0.67–0.94)	0.67 (0.55–0.81)	0.00003
22:0						
Cases/person-years	312/8850	310/9407	257/9322	239/8818	186/8632	
Incidence rate, ‰	3.52%	3.30%	2.76%	2.71%	2.15%	
Demographics adjusted*	REF	0.95 (0.81–1.11)	0.81 (0.69–0.96)	0.82 (0.69–0.97)	0.71 (0.59–0.86)	0.00006
Multivariable adjusted*	REF	0.99 (0.85–1.16)	0.90 (0.76–1.06)	0.90 (0.76–1.07)	0.80 (0.66–0.96)	0.01
Multivariable+FA adjusted*	REF	0.96 (0.82–1.12)	0.84 (0.71–0.99)	0.83 (0.70–0.99)	0.72 (0.60–0.87)	0.0002
20:0						
Cases/person-years	237/7377	262/8868	258/8715	269/9727	278/10 342	
Incidence rate, ‰	3.21%	2.95%	2.96%	2.77%	2.69%	
Demographics adjusted*	REF	0.87 (0.73–1.04)	0.88 (0.74–1.05)	0.80 (0.67–0.95)	0.80 (0.67–0.95)	0.009
Multivariable adjusted*	REF	0.97 (0.81–1.16)	0.99 (0.83–1.18)	0.94 (0.78–1.12)	0.93 (0.78–1.11)	0.36
Multivariable+FA adjusted*	REF	0.90 (0.75–1.07)	0.87 (0.73–1.05)	0.79 (0.65–0.95)	0.72 (0.59–0.88)	0.0007

20:0 indicates arachidic acid; 22:0, behenic acid; 24:0, lignoceric acid; FA, fatty acids; REF, reference.
*The table shows hazard ratios of heart failure associated with quintiles of circulating very-long-chain saturated fatty acids compared with the lowest quintile, with different levels of adjustments. Demographics model adjusted for age, sex, race, and clinic site. Multivariable model further adjusted for prevalent coronary heart disease, atrial fibrillation, diabetes mellitus, fasting glucose levels, treated hypertension, systolic blood pressure, body mass index, waist circumference, smoking status, and physical activity. Multivariable+fatty acids (FA) model further adjusted for circulating levels of phospholipid fatty acids 20:5n3 (eicosapentaenoic acid) and 24:1n9 (nervonic acid).

likely to have diabetes mellitus, coronary heart disease, atrial fibrillation, and hypertension, and more likely to have higher low-density lipoprotein cholesterol and lower fasting triglycerides.

During 45 030 person-years, we identified 1304 cases of incident HF, including 489 HFpEF, 310 HFrEF, and 505 with unknown classification. After multivariable adjustment for demographics and major HF risk factors, higher levels of 22:0 and 24:0, but not 20:0, were each associated with lower risk of incident HF (Table 3). For example, the HR in the upper quintile compared with the lowest quintile was 0.73 (95% confidence interval [CI], 0.61–0.88) for 24:0 (*P* trend, 0.001) and 0.80 (95% CI, 0.66–0.96) for 22:0 (*P* trend, 0.01). Additional adjustment for 2 plasma phospholipid FAs known to be associated with HF in the CHS[18,20] strengthened these associations, with higher levels of 20:0 now also significantly associated with lower risk (*P* trend=0.0007). Further adjustment for plasma phospholipid palmitic acid (16:0) or blood levels of low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, or triglycerides had minimal impact on these associations (not shown).

In continuous linear analyses evaluating each VLSFA according to its interquintile range, after multivariable+FA adjustment, the HR associated with 24:0 was 0.76 (95% CI, 0.65–0.89; *P*=0.001) and similarly for 22:0 (HR, 0.77; 95% CI, 0.66–0.90; *P*=0.001) and 20:0 (HR, 0.71; 95% CI, 0.60–0.84; $P=8\times10^{-5}$). In further analyses that examined all 3 VLSFAs jointly in the same model, both 24:0 and 20:0, but not 22:0, were associated with lower risk of HF, with interquintile range HRs (95% CI) of 0.65 (0.46–0.94) for 24:0 and 0.69 (0.54–0.87) for 20:0. Adjusted for 24:0 and 20:0, the HR for 22:0 was 1.40 (0.91–2.14), possibly attributable to noise.

Data on ejection fraction measurements were available for 60% of HF cases. In secondary analyses, we examined the association of VLSFA levels with HF with and without preserved ejection fraction (Figure). Higher levels of 24:0 and 22:0 were associated with lower risk of incident HFpEF, but not HFrEF, although point estimates for the HRs were generally similar and did not differ significantly (Figure and Table S1).

In exploratory analyses, we did not find evidence of interaction between the VLSFAs and sex, age, BMI, diabetes

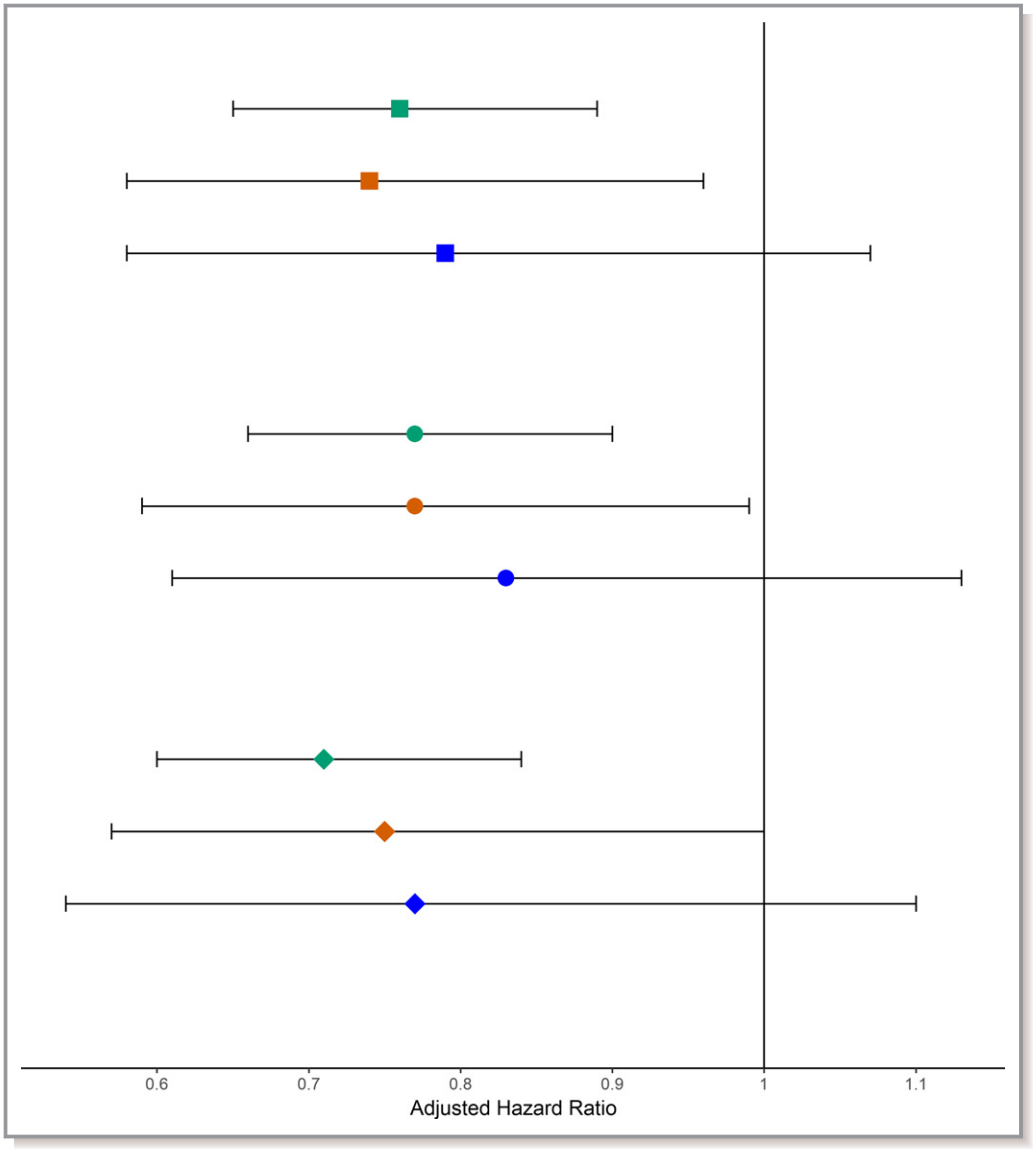


Figure. Associations of very-long-chain saturated fatty acids with total incident heart failure, heart failure with preserved ejection fraction, and heart failure with low ejection fraction. Hazard ratios of total incident heart failure (green), heart failure with preserved ejection fraction (red), and heart failure with reduced ejection fraction (blue), associated with higher levels of 24:0 (top estimates), 22:0 (middle estimates), and 20:0 (bottom estimates) corresponding to the interquintile range. Multivariate+fatty acids (FA) adjustment models as in Table 2. 20:0 indicates arachidic acid; 22:0, behenic acid; 24:0, lignoceric acid.

mellitus, and coronary heart disease in relation to risk of HF (*P* values for interaction, ≥0.10 each), shown for 24:0 in Table S2.

Discussion

In this large, prospective cohort of older US adults, we have identified, for the first time to our knowledge, an association of higher levels of plasma phospholipid VLSFA with lower risk of incident HF. The associations were independent of major HF risk factors and other FAs and did not significantly differ by sex, age, BMI, diabetes mellitus, and coronary heart disease. When the VLSFAs were evaluated jointly, 24:0 and 20:0, but not 22:0, were independently associated with lower risk of HF. The associations of the VLSFA with HFpEF and HFrEF were similar to the associations with total HF.

Higher levels of circulating VLSFAs have been associated with lower risk of atrial fibrillation in the CHS[13] and lower risk of coronary heart disease in the Harvard cohorts.[14] In the present study, the VLSFA associations with HF were independent of time-dependent adjustment for these risk factors, suggesting that atrial fibrillation and coronary heart disease did not mediate the inverse associations with HF. The VLSFAs are also associated with lower risk of diabetes mellitus in the CHS and in the InterAct Study,[11,12] and these associations

appear, at least in part, to be mediated by circulating levels of triglycerides and 16:0, markers of lipogenesis and FA synthesis. In the current study, the VLSFA associations with HF were independent of levels of fasting triglycerides and 16:0, suggesting that potential mechanisms underlying the associations of VLSFA with HF versus diabetes mellitus may differ.

The VLSFAs are known components of ceramides and other sphingolipids,[22] and ceramides are involved in apoptosis and heart dysfunction.[23] However, unlike ceramides with palmitic acid, cell and animal studies provide strong evidence that ceramides that carry a VLSFA actually protect against apoptosis.[9,24–26] For example, the heart of genetically engineered mice with reduced ceramides containing VLSFA showed increased fibrosis, endoplasmic reticulum stress, and apoptosis.[26] Further studies are needed to investigate whether ceramides with VLSFA play a role in the prevention of HF in humans.

Levels of circulating VLSFA are influenced by both diet and metabolism. For example, VLSFAs are found in certain foods, including peanuts, macadamia nuts, and canola oil.[27] Small short-term feeding trials show that consumption of macadamia nuts[28] and peanut butter[29] increase circulating levels of VLSFA. In the CHS, levels of 22:0 and 24:0 are also associated with peanut consumption.[11] However, VLSFAs can also be synthesized endogenously from shorter-chain saturated FAs by the action of elongases.[30,31] In particular, the ubiquitous elongase, elovl1, elongates stearic acid (18 carbons) to 20:0 and longer VLSFA.[30] Furthermore, elovl1 appears to be coupled to ceramide synthase 2 and to provide 24:0 for the synthesis of sphingolipids with 24:0.[32] In agreement with a role of VLSFA in sphingolipid metabolism, we showed, in a genome-wide association study, that circulating levels of VLSFA were associated with variation in genes involved in de novo synthesis of sphingolipids.[33] Overall, the relative contribution of diet versus metabolism to circulating levels of VLSFA, and whether it differs for 20:0, 22:0, and 24:0, is not known. The associations observed in the present investigation suggest that both dietary and metabolic influences, either of which may alter circulating VLSFA levels, may potentially influence the risk of HF.

Strengths of the study include the prospective design, population-based cohort, use of an objective marker of diet and metabolism, repeated VLSFA measurements, and information on multiple potential risk factors. The study also has potential limitations. This is an observational study, and causality cannot be conclusively established. Confounding by unknown factors is also possible. However, results were robust to adjustment for multiple major HF risk factors. The study was conducted among older adults, the population at highest risk of HF, and results may not be generalizable to younger populations. Although the total number of HF cases and HFpEF cases were large, fewer cases of HFrEF occurred, potentially limiting power to confirm associations of VLSFA with HFrEF.

In conclusion, we report novel associations of higher levels of plasma phospholipid VLSFA with lower risk of incident HF among older adults. The study findings should prompt further research on the role of VLSFA in HF and the determinants of circulating levels of VLSFA.

Sources of Funding

This research was supported by R01-HL085710 and R01-HL094555 from the National Heart, Lung, and Blood Institute (NHLBI), with co-funding from the Office of Dietary Supplements. The CHS Cohort was supported by contracts HHSN268201200036C, HHSN268200800007C, HHSN26 8201800001C, N01HC55222, N01HC85079, N01HC85080, N01HC85081, N01HC85082, N01HC85083, N01HC85086, and grants U01HL080295 and U01HL130114 from the NHLBI, with additional contribution from the National Institute of Neurological Disorders and Stroke (NINDS). Additional support was provided by R01AG023629 from the National Institute on Aging (NIA). A full list of principal CHS investigators and institutions can be found at CHS-NHLBI.org. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Disclosures

Dr Psaty serves on the DSMB of a clinical trial funded by Zoll LifeCor and on the Steering Committee of the Yale Open Data Access Project funded by Johnson & Johnson. Dr Mozaffarian reports research funding from the National Institutes of Health and the Gates Foundation; personal fees from GOED, DSM, Nutrition Impact, Pollock Communications, Bunge, Indigo Agriculture, Amarin, Acasti Pharma, and America's Test Kitchen; scientific advisory board, Omada Health, Elysium Health, and DayTwo; and chapter royalties from UpToDate; all outside the submitted work. The remaining authors have no disclosures to report.

References

1. Benjamin EJ, Blaha MJ, Chiuve SE, Cushman M, Das SR, Deo R, de Ferranti SD, Floyd J, Fornage M, Gillespie C, Isasi CR, Jimenez MC, Jordan LC, Judd SE, Lackland D, Lichtman JH, Lisabeth L, Liu S, Longenecker CT, Mackey RH, Matsushita K, Mozaffarian D, Mussolino ME, Nasir K, Neumar RW, Palaniappan L, Pandey DK, Thiagarajan RR, Reeves MJ, Ritchey M, Rodriguez CJ, Roth GA, Rosamond WD, Sasson C, Towfighi A, Tsao CW, Turner MB, Virani SS, Voeks JH, Willey JZ, Wilkins JT, Wu JH, Alger HM, Wong SS, Muntner P; American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics—2017 update: a report from the American Heart Association. *Circulation*. 2017;135:e146–e603.
2. Gottdiener JS, McClelland RL, Marshall R, Shemanski L, Furberg CD, Kitzman DW, Cushman M, Polak J, Gardin JM, Gersh BJ, Aurigemma GP, Manolio TA. Outcome of congestive heart failure in elderly persons: influence of left ventricular systolic function. The cardiovascular health study. *Ann Intern Med*. 2002;137:631–639.
3. Bui AL, Horwich TB, Fonarow GC. Epidemiology and risk profile of heart failure. *Nat Rev Cardiol*. 2011;8:30–41.

4. Ammar KA, Jacobsen SJ, Mahoney DW, Kors JA, Redfield MM, Burnett JC Jr, Rodeheffer RJ. Prevalence and prognostic significance of heart failure stages: application of the American College of Cardiology/American Heart Association Heart Failure Staging Criteria in the Community. *Circulation*. 2007;115:1563–1570.
5. Gault CR, Obeid LM, Hannun YA. An overview of sphingolipid metabolism: from synthesis to breakdown. *Adv Exp Med Biol*. 2010;688:1–23.
6. Pettus BJ, Chalfant CE, Hannun YA. Ceramide in apoptosis: an overview and current perspectives. *Biochem Biophys Acta*. 2002;1585:114–125.
7. Li XM, Ma YT, Yang YN, Liu F, Chen BD, Han W, Zhang JF, Gao XM. Downregulation of survival signalling pathways and increased apoptosis in the transition of pressure overload-induced cardiac hypertrophy to heart failure. *Clin Exp Pharmacol Physiol*. 2009;36:1054–1061.
8. Abbate A, Biondi-Zoccai GG, Bussani R, Dobrina A, Camilot D, Feroce F, Rossiello R, Baldi F, Silvestri F, Biasucci LM, Baldi A. Increased myocardial apoptosis in patients with unfavorable left ventricular remodeling and early symptomatic post-infarction heart failure. *J Am Coll Cardiol*. 2003;41:753–760.
9. Grosch S, Schiffmann S, Geisslinger G. Chain length-specific properties of ceramides. *Prog Lipid Res*. 2012;51:50–62.
10. Stiban J, Perera M. Very long chain ceramides interfere with c16-ceramide-induced channel formation: a plausible mechanism for regulating the initiation of intrinsic apoptosis. *Biochem Biophys Acta*. 2015;1848:561–567.
11. Lemaitre RN, Fretts AM, Sitlani CM, Biggs ML, Mukamal K, King IB, Song X, Djousse L, Siscovick DS, McKnight B, Sotoodehnia N, Kizer JR, Mozaffarian D. Plasma phospholipid very-long-chain saturated fatty acids and incident diabetes in older adults: the cardiovascular health study. *Am J Clin Nutr*. 2015;101:1047–1054.
12. Forouhi NG, Koulman A, Sharp SJ, Imamura F, Kroger J, Schulze MB, Crowe FL, Huerta JM, Guevara M, Beulens JW, van Woudenbergh GJ, Wang L, Summerhill K, Griffin JL, Feskens EJ, Amiano P, Boeing H, Clavel-Chapelon F, Dartois L, Fagherazzi G, Franks PW, Gonzalez C, Jakobsen MU, Kaaks R, Key TJ, Khaw KT, Kuhn T, Mattiello A, Nilsson PM, Overvad K, Pala V, Palli D, Quiros JR, Rolandsson O, Roswall N, Sacerdote C, Sanchez MJ, Slimani N, Spijkerman AM, Tjonneland A, Tormo MJ, Tumino R, van der A DL, van der Schouw YT, Langenberg C, Riboli E, Wareham NJ. Differences in the prospective association between individual plasma phospholipid saturated fatty acids and incident type 2 diabetes: the epic-interact case-cohort study. *Lancet Diabetes Endocrinol*. 2014;2:810–818.
13. Fretts AM, Mozaffarian D, Siscovick DS, Djousse L, Heckbert SR, King IB, McKnight B, Sitlani C, Sacks FM, Song X, Sotoodehnia N, Spiegelman D, Wallace ER, Lemaitre RN. Plasma phospholipid saturated fatty acids and incident atrial fibrillation: the cardiovascular health study. *J Am Heart Assoc*. 2014;3:e000889. DOI: 10.1161/JAHA.114.000889.
14. Malik VS, Chiuve SE, Campos H, Rimm EB, Mozaffarian D, Hu FB, Sun Q. Circulating very-long-chain saturated fatty acids and incident coronary heart disease in US men and women. *Circulation*. 2015;132:260–268.
15. Fried LP, Borhani NO, Enright P, Furberg CD, Gardin JM, Kronmal RA, Kuller LH, Manolio TA, Mittelmark MB, Newman A, O'Leary DH. The cardiovascular health study: design and rationale. *Ann Epidemiol*. 1991;1:263–276.
16. Folch J, Lees M, Sloane GH. A simple method for isolation and purification of total lipids from animal tissues. *J Biol Chem*. 1957;226:497–509.
17. Lepage G, Roy CC. Direct transesterification of all lipids in a one-step reaction. *J Lipid Res*. 1986;27:114–120.
18. Mozaffarian D, Lemaitre RN, King IB, Song X, Spiegelman D, Sacks FM, Rimm EB, Siscovick DS. Circulating long-chain {omega}-3 fatty acids and incidence of congestive heart failure in older adults: the cardiovascular health study: a cohort study. *Ann Intern Med*. 2011;155:160–170.
19. Gottdiener JS, Arnold AM, Aurigemma GP, Polak JF, Tracy RP, Kitzman DW, Gardin JM, Rutledge JE, Boineau RC. Predictors of congestive heart failure in the elderly: the cardiovascular health study. *J Am Coll Cardiol*. 2000;35:1628–1637.
20. Imamura F, Lemaitre RN, King IB, Song X, Steffen LM, Folsom AR, Siscovick DS, Mozaffarian D. Long-chain monounsaturated fatty acids and incidence of congestive heart failure in 2 prospective cohorts. *Circulation*. 2013;127:1512–1521.
21. Lunn M, McNeil D. Applying Cox regression to competing risks. *Biometrics*. 1995;51:524–532.
22. Ohno Y, Suto S, Yamanaka M, Mizutani Y, Mitsutake S, Igarashi Y, Sassa T, Kihara A. Elovl1 production of c24 acyl-coas is linked to c24 sphingolipid synthesis. *Proc Natl Acad Sci USA*. 2010;107:18439–18444.
23. Park TS, Hu Y, Noh HL, Drosatos K, Okajima K, Buchanan J, Tuinei J, Homma S, Jiang XC, Abel ED, Goldberg IJ. Ceramide is a cardiotoxin in lipotoxic cardiomyopathy. *J Lipid Res*. 2008;49:2101–2112.
24. Deng X, Yin X, Allan R, Lu DD, Maurer CW, Haimovitz-Friedman A, Fuks Z, Shaham S, Kolesnick R. Ceramide biogenesis is required for radiation-induced apoptosis in the germ line of *C. elegans*. *Science*. 2008;322:110–115.
25. Crowder CM. Cell biology. Ceramides—friend or foe in hypoxia? *Science*. 2009;324:343–344.
26. Lee SY, Kim JR, Hu Y, Khan R, Kim SJ, Bharadwaj KG, Davidson MM, Choi CS, Shin KO, Lee YM, Park WJ, Park IS, Jiang XC, Goldberg IJ, Park TS. Cardiomyocyte specific deficiency of serine palmitoyltransferase subunit 2 reduces ceramide but leads to cardiac dysfunction. *J Biol Chem*. 2012;287:18429–18439.
27. US Department of Agriculture Agricultural Research Service. USDA National Nutrient Database for Standard Reference, Release 26. Nutrient data laboratory home page. 2013. Available at: http://www.Ars.Usda.Gov/ba/bhnrc/ndl. Accessed March 26, 2018.
28. Garg ML, Blake RJ, Wills RB. Macadamia nut consumption lowers plasma total and LDL cholesterol levels in hypercholesterolemic men. *J Nutr*. 2003;133:1060–1063.
29. Lam C, Wong D, Cederbaum S, Lim B, Qu Y. Peanut consumption increases levels of plasma very long chain fatty acids in humans. *Mol Genet Metab*. 2012;107:620–622.
30. Kihara A. Very long-chain fatty acids: elongation, physiology and related disorders. *J Biochem*. 2012;152:387–395.
31. Jakobsson A, Westerberg R, Jacobsson A. Fatty acid elongases in mammals: their regulation and roles in metabolism. *Prog Lipid Res*. 2006;45:237–249.
32. Sassa T, Kihara A. Metabolism of very long-chain fatty acids: genes and pathophysiology. *Biomol Ther (Seoul)*. 2014;22:83–92.
33. Lemaitre RN, King IB, Kabagambe EK, Wu JH, McKnight B, Manichaikul A, Guan W, Sun Q, Chasman DI, Foy M, Wang L, Zhu J, Siscovick DS, Tsai MY, Arnett DK, Psaty BM, Djousse L, Chen YD, Tang W, Weng LC, Wu H, Jensen MK, Chu AY, Jacobs DR Jr, Rich SS, Mozaffarian D, Steffen L, Rimm EB, Hu FB, Ridker PM, Fornage M, Friedlander Y. Genetic loci associated with circulating levels of very long-chain saturated fatty acids. *J Lipid Res*. 2015;56:176–184.

SUPPLEMENTAL MATERIAL

Table S1. Associations of plasma phospholipid very long-chain saturated fatty acid levels with total incident heart failure, heart failure with preserved ejection fraction and heart failure with low ejection fraction

	Total Heart Failure 1304 cases		**Heart failure with preserved ejection fraction** 489 cases		**Heart failure with reduced ejection fraction** 310 cases		
	HR* (95% CI)	**p**	**HR* (95% CI)**	**p**	**HR* (95% CI)**	**p**	**P for difference between outcomes¥**
24:0	0.76 (0.65-0.89)	0.001	0.74 (0.58-0.96)	0.02	0.79 (0.58-1.07)	0.13	0.79
22:0	0.77 (0.66-0.90)	0.001	0.77 (0.59-0.99)	0.04	0.83 (0.61-1.13)	0.23	0.71
20:0	0.71 (0.60-0.84)	$8x10^{-5}$	0.75 (0.57-1.00)	0.05	0.77 (0.54-1.10)	0.15	0.93

*Hazard ratio (HR) for the interquintile range, the difference between the midpoints of the top and bottom quintiles (90^{th} and 10^{th} percentiles) of fatty acid levels.

Analyses adjusted for age, sex, race, clinic site, prevalent coronary heart disease, atrial fibrillation, diabetes, fasting glucose levels, treated hypertension, systolic blood pressure, body mass index, waist circumference, smoking status and physical activity, and circulating levels of phospholipid fatty acids 20:5n3 (eicosapentaenoic acid) and 24:1n9 (nervonic acid).

¥Formal tests for a difference in hazard ratios of heart failure with preserved ejection fraction vs heart failure with reduced ejection fraction

20:0 = arachidic acid; 22:0 = behenic acid; 24:0 = lignoceric acid

Table S2. Associations of levels of plasma phospholipid 24:0 with incident heart failure in stratified analyses.

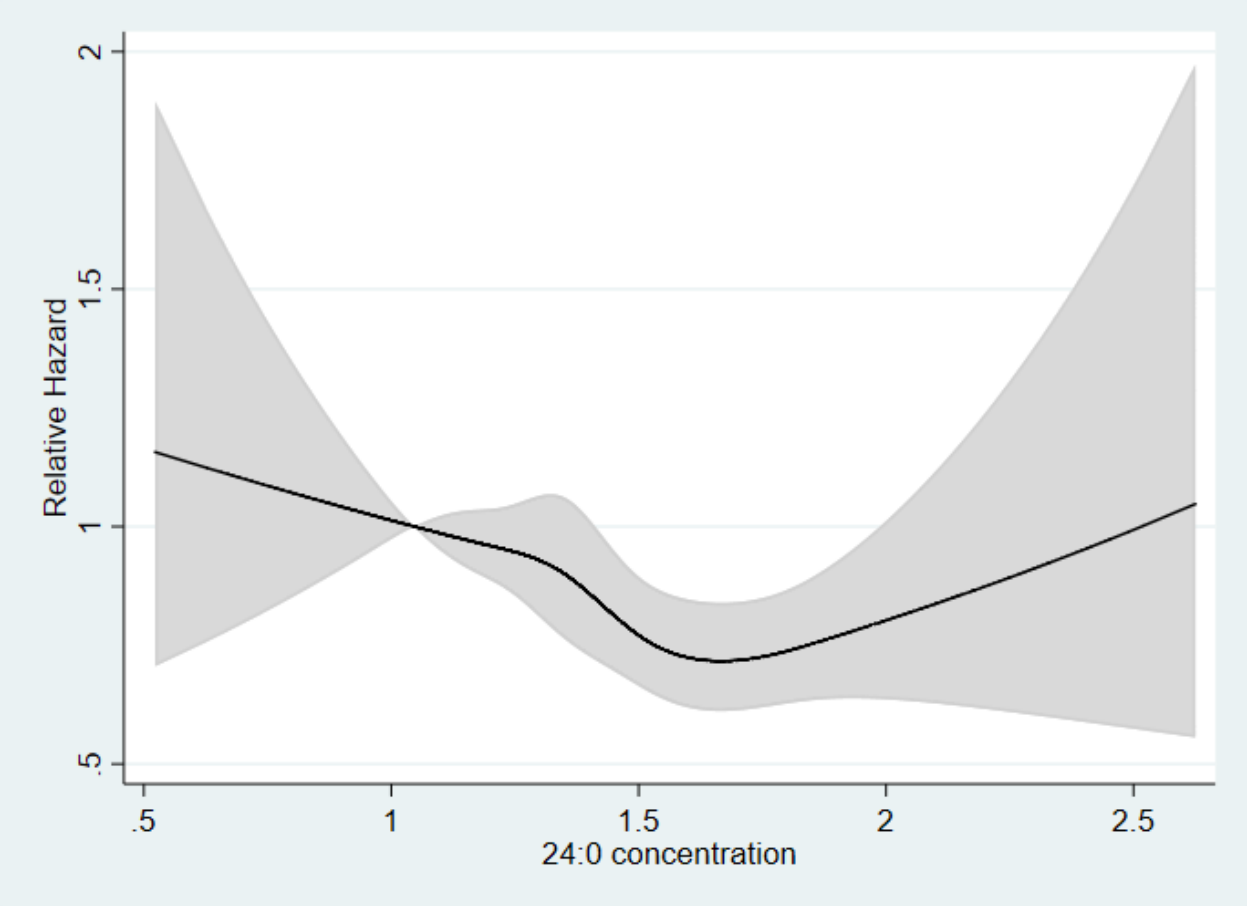
Stratum	# HF cases	Hazard ratio	95% Confidence Interval	P for interaction
Age < 77	426	0.77	0.60 – 0.99	0.15
Age ≥ 77	878	0.74	0.61 – 0.90	
Men	578	0.77	0.61 – 0.96	0.96
Women	726	0.78	0.64 – 0.97	
CHD =0	900	0.80	0.67 – 0.97	0.48
CHD =1	404	0.69	0.52 – 0.91	
BMI ≤ 26	596	0.82	0.65 – 1.03	0.10
BMI > 26	708	0.73	0.60 – 0.90	
Diabetes = 0	1013	0.79	0.66 – 0.95	0.55
Diabetes = 1	291	0.73	0.42 – 0.99	

Hazard ratio (HR) for a difference between 90^{th} and 10^{th} percentiles of 24:0.

Model adjusted for age, sex, race, clinic site, coronary heart disease, atrial fibrillation, diabetes, fasting glucose levels, treated hypertension, systolic blood pressure, body mass index, waist circumference, smoking status and physical activity, circulating levels of phospholipid fatty acids 20:5n3 (eicosapentaenoic acid) and 24:1n9 (nervonic acid).

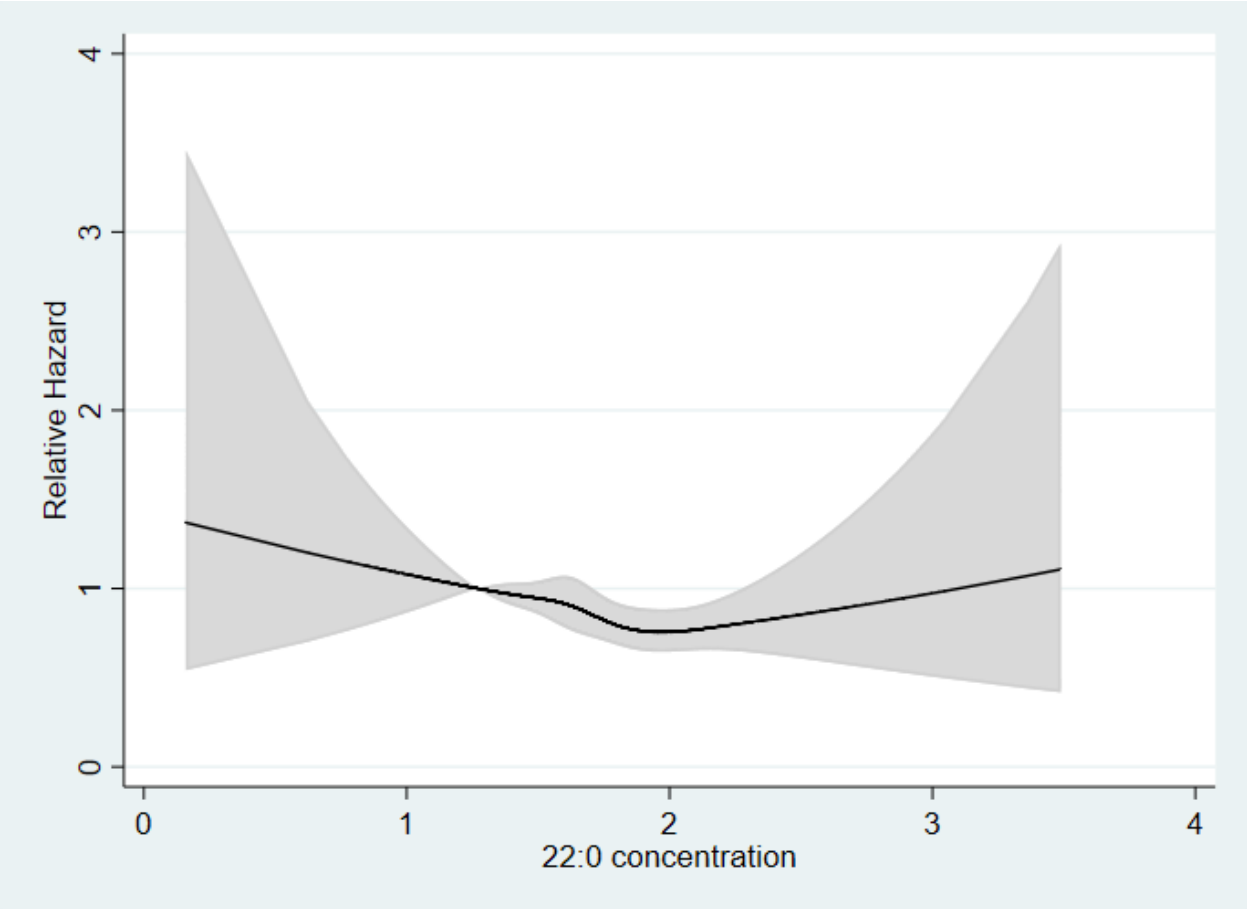
24:0= lignoceric acid; CHD=coronary heart disease; BMI=body mass index

Figure S1. Association of levels of plasma phospholipid 24:0 with risk of incident heart failure – cubic spline analysis.



The figure shows estimated hazard ratios (black line) and point-wise 95% confidence intervals (shaded area) for the association between incident heart failure and plasma phospholipid concentration of 24:0, relative to the 10th percentile of 24:0 (1.046). Concentration of 24:0 is expressed as % of total plasma phospholipid fatty acids. Analyses adjusted for age, sex, race, clinic site, prevalent coronary heart disease, atrial fibrillation, diabetes, fasting glucose levels, treated hypertension, systolic blood pressure, body mass index, waist circumference, smoking status and physical activity, and circulating levels of phospholipid fatty acids 20:5n3 (eicosapentaenoic acid) and 24:1n9 (nervonic acid). Quintile cut-offs for 24:0 were 1.153, 1.302, 1.435 and 1.612. 24:0=lignoceric acid

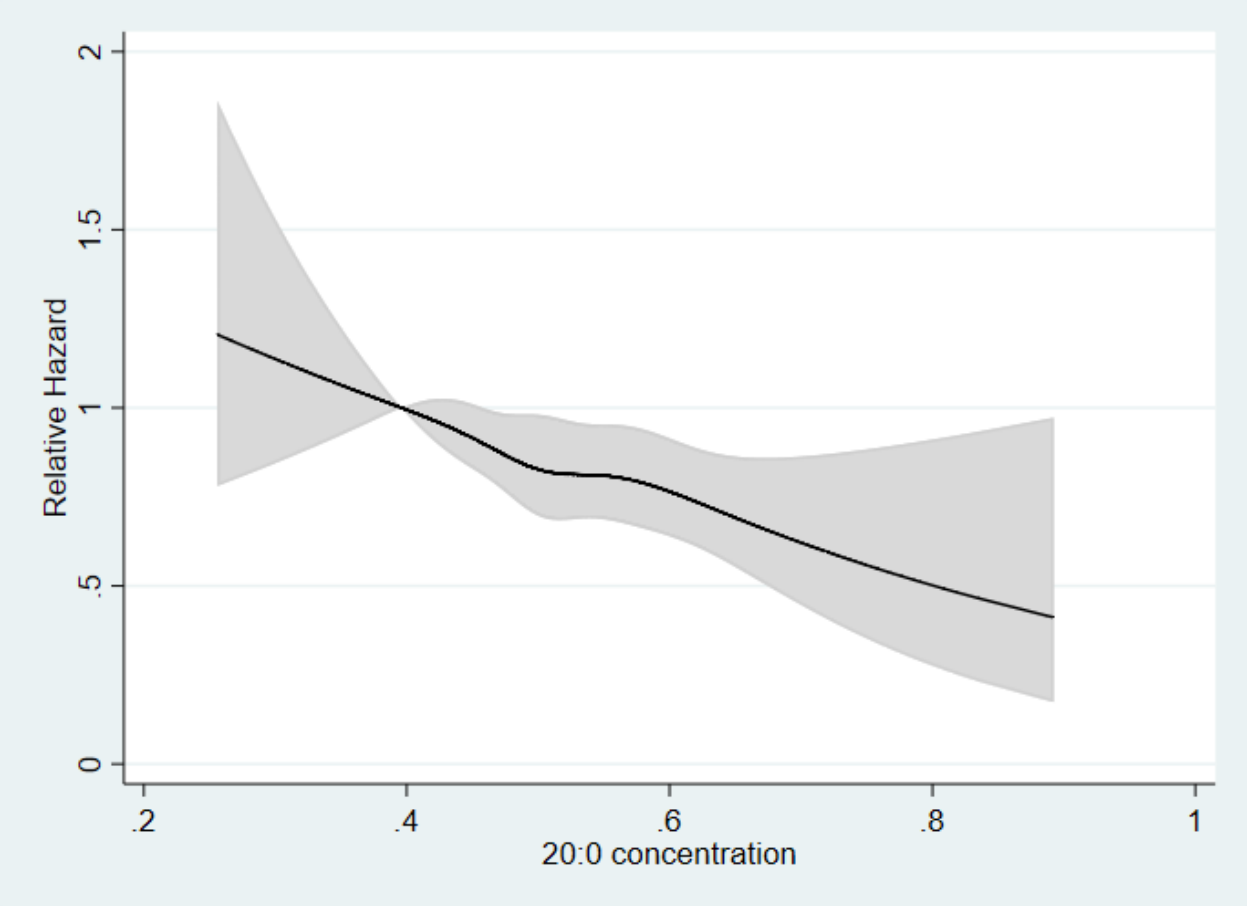
Figure S2. Association of levels of plasma phospholipid 22:0 with risk of incident heart failure – cubic spline analysis.



The figure shows hazard ratios of incident heart failure (black line), and point-wise 95% confidence intervals (shaded area) associated with 22:0, relative to the 10th percentile of 22:0 (1.27). Concentration of 22:0 is as % of total fatty acids. Analyses adjusted as in figure S1. Quintile cut-offs for 22:0 were 1.406, 1.573, 1.727 and 1.914.

22:0=behenic acid

Figure S3. Association of levels of plasma phospholipid 20:0 with risk of incident heart failure – cubic spline analysis.



The figure shows hazard ratios of incident heart failure (black line), and point-wise 95% confidence intervals (shaded area) associated with 20:0, relative to the 10th percentile of 20:0 (0.396). Concentration of 20:0 is as % of total fatty acids. Analyses adjusted as in figure S1. Quintile cut-offs for 20:0 were 0.429, 0.477, 0.515, and 0.565.

20:0=arachidic acid.