Associations of Serum Nonesterified Fatty Acids With Coronary Heart Disease Mortality and Nonfatal Myocardial Infarction: The CHS (Cardiovascular Health Study) Cohort

Neil K. Huang, PhD; Petra Bůžková, PhD; Nirupa R. Matthan, PhD; Luc Djoussé, MD, MPH, DSc; Calvin H. Hirsch, MD; Jorge R. Kizer, MD, MSc; W. T. Longstreth, Jr, MD, MPH; Kenneth J. Mukamal, MD, MPH; Alice H. Lichtenstein, DSc

BACKGROUND: Significant associations have been reported between serum total nonesterified fatty acid (NEFA) concentrations and coronary heart disease (CHD) mortality and incident nonfatal myocardial infarction (MI) in some prospective cohort studies. Little is known about whether individual or subclasses (saturated, polyunsaturated [n-6 and n-3], and trans fatty acids) of serum NEFAs relate to CHD mortality and nonfatal MI.

METHODS AND RESULTS: CHS (Cardiovascular Health Study) participants (N=1681) who had no history of MI, angina, or revascularization or were free of MI at baseline (1996–1997) were included. NEFAs were quantified using gas chromatography. Cox regression analysis was used to evaluate associations of 5 subclasses and individual NEFAs with CHD composite (CHD mortality and nonfatal MI), CHD mortality, and incident nonfatal MI. During a median follow-up of 11.7 years, 266 cases of CHD death and 271 cases of nonfatal MI occurred. In the fully adjusted model, no significant associations were identified between individual NEFA and CHD composite. Exploratory analyses indicated that lauric acid (12:0) was negatively associated (hazard ratio [HR], 0.76; 95% CI, 0.59–0.98; P=0.0328) and dihomo-γ-linolenic acid (20:3n-6) was positively associated with CHD mortality (HR, 1.34; 95% CI, 1.02–1.76; P=0.0351). Elaidic acid (18:1n-7t) was positively associated with incident nonfatal MI (HR, 1.46; 95% CI, 1.01–2.12; P=0.0445). No significant associations were observed for NEFA subclass and any outcomes.

CONCLUSIONS: In CHS participants, 2 NEFAs, dihomo-γ-linolenic and elaidic acids, were positively associated with CHD mortality and nonfatal MI, respectively, suggesting potential susceptibility biomarkers for risks of CHD mortality and nonfatal MI.

Key Words: coronary heart disease mortality ■ dihomo-γ-linolenic acid ■ epidemiology ■ incident nonfatal myocardial infarction ■ serum nonesterified fatty acid ■ trans fat

Cardiovascular disease, including coronary heart disease (CHD), is the leading cause of morbidity and mortality in the United States. By 2030, the medical costs associated with CHD are estimated to double from current levels.1 Inflammation and disorders of lipid metabolism are linked to coronary artery plaque initiation and progression.2–4 Elevated concentrations of nonesterified fatty acids (NEFA) have been associated with increased local and systemic inflammation and induction of oxidative stress,5 insulin resistance,6,7 endothelial dysfunction,8 and foam cell formation.5,6,8
Data from cross-sectional and prospective studies identified significant positive associations between plasma total NEFA concentrations and CHD risk factors, including hypertension, obesity, diabetes mellitus, insulin resistance, and systemic inflammation. Total plasma NEFA has also been linked to adverse cardiovascular events. In the CHS (Cardiovascular Health Study) cohort, plasma total NEFA concentrations were positively associated with heart failure but not ischemic stroke and cardiac arrest. In other cohorts plasma total NEFA concentrations have been associated with severity of coronary artery disease. However, lacking is an assessment of the relation between NEFA subclasses (saturated FA, omega-6 polyunsaturated [PUFA], n-3 PUFA, and trans fatty acid [FA]) or individual NEFAs and CHD risk in a large population of older adults. Preliminary support for the importance of resolving this uncertainty comes from data for other plasma circulating lipid subfractions. Specifically, plasma long-chain phospholipid (PL) saturated and trans FAs have been positively associated with incident CHD, whereas both plasma PL total n-6 and n-3 PUFA, as well as 3 individual plasma long-chain PL FAs (linoleic acid, eicosapentaenoic acid, and docosahexaenoic acid), have been negatively associated with CHD risk.

The objective of this study was to assess the relation between serum NEFA subclasses and individual NEFAs, and a composite outcome of CHD (CHD composite, including CHD mortality and nonfatal myocardial infarction [MI]), CHD mortality and nonfatal MI in CHS participants. We hypothesized that fasting serum nonesterified n-3 and n-6 PUFAs, either as a subclass or individually, would be inversely associated, and nonesterified saturated FA and trans FA, either as a subclass or individually, would be positively associated with CHD composite, CHD mortality, and incident nonfatal MI.
used during sample collection. Prior work has demonstrated that plasma phospholipids, cholesteryl ester, and triglyceride fatty acid profiles were stable for at least 10 years when stored at −80°C. Lipids were extracted from serum using a modified Folch method after addition of an internal standard (heptadecanoic acid). The serum NEFA fraction was isolated using solid-phase chromatography (amino-propyl columns), saponified, methylated, and the resulting fatty acid methyl esters were quantified using an Autosystem XL gas chromatograph (Perkin Elmer, Boston, MA) equipped with a 100 m×0.25 mm capillary column (HP INNOWQAX, Agilent Technologies, DE) as previously described. Thirty-five individual FAs were identified by comparison with authenticated standards (NuCheck Prep, MN), and are reported as absolute concentrations (µmol/L). The adjustments were made by including a pooled human serum sample as a quality control standard at the beginning and the middle of each run (81 samples/run) for quality control purposes. The lower limits of quantification of the NEFA assay is 0.01% total fatty acid (% weight). The intra- and interassay coefficients of variation were 0.5% to 4.3% for FAs present at concentrations >25 µmol/L, 1.8% to 7.1% for FAs present at concentrations between 5 and 25 µmol/L, and 2.8% to 11.1% for FAs present at concentrations <5 µmol/L. The NEFA analysis was conducted at the Jean Mayer US Department of Agriculture Human Nutrition Research Center on Aging at Tufts University, Boston, MA, USA.

Follow-Up and Ascertainment of CHD Composite, CHD Mortality, and MI

Surveillance for cardiovascular events occurred during annual clinic visits and intervening 6-month telephone contacts through 1999 and thereafter by twice yearly telephone contacts through June 2015. At each 6-month interval participants were contacted to request updates on new cardiovascular events and hospitalizations. Medicare data were used to verify cardiovascular events and hospitalizations. Medicare data were collected using standardized protocols. Other Covariates

Age, sex, race, and educational level were reported by participants at enrollment. All participant characteristics were collected at the 1996 to 1997 visit, which serves as baseline for the current analysis. Smoking status (never, former, and current), alcohol intake (none, 1–6 drinks/week, 7–14 drinks/week, >14 drinks/week), and health status were ascertained by questionnaire. Weight, height, waist circumference, fasting glucose, serum albumin, and C-reactive protein were measured using standard methods. Physical activity (quantified as metabolic equivalents per week) was assessed using Minnesota Leisure-Time Activities questionnaire. Renal function was assessed based on cystatin C for estimated glomerular filtration rate (mL/min per 1.73 m²). Information regarding diabetes mellitus (defined as fasting glucose ≥7 mmol/L [126 mg/dL], oral glucose tolerance test ≥11.1 mmol/L [200 mg/dL], or use of oral hypoglycemic medications or insulin) and hypertension (defined as systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, or treatment with blood pressure lowering medications plus reported physician diagnosis of hypertension) were collected using standardized protocols.

Statistical Analysis

To characterize the study population at the analysis baseline, we calculated means and SDs for continuous measures and percentages for binary and categorical variables. Five NEFA subclasses and individual NEFA were expressed as absolute serum concentration (µmol/L). The adjustments were made by inclusions of all 5 NEFA subclasses for NEFA subclasses analysis or all 35 NEFAs for individual NEFA analysis in the Cox regression models, without the need for correction of multiple testing. The subclasses and the individual NEFAs were standardized and scaled as association with CHD mortality and nonfatal MI per 1 SD. Cox proportional hazards regression was used to estimate the risk of CHD composite, CHD mortality, and incident nonfatal MI. Time to first event or censoring was calculated as the time from the date of the 1996 to 1997 study visit to the earliest date of nonfatal MI or CHD, date of death, date of loss to follow-up, or date of administrative censoring (June 2015). The NEFA subclasses and individual NEFAs were used for the primary and secondary analyses, respectively.

Multivariable analyses were adjusted in nested models as follows: model 1—age, sex, race, field center,
education, and other NEFA subclasses for the primary analysis or all other 35 NEFAs for the secondary analysis; model 2—all the covariates in model 1 plus weight, height, smoking, physical activity, serum albumin, alcohol intake, and renal function; and model 3—all the covariates in the model 2 plus hypertension and diabetes mellitus.

All analyses were conducted in R software (version 3.6.3; Vienna, Austria) using survival and ggplot2 packages, and statistical significance was defined as 2-tailed $\alpha \leq 0.05$.

RESULTS

Characteristics of Study Participants

The mean age of the 1681 participants at year 9 was 77.6±4.4 years, body mass index was 26.7±4.4, and waist circumference was 96.2±12.9 (Table 1). Of the participants, over one-third were male, one-seventh were Black, and approximately three-fifths were diagnosed with hypertension. The participants were relatively young and healthy, compared with those who were not included (Table S1).

The most abundant serum individual NEFAs ($\mu$mol/L) in the CHS participants were oleic acid (153±63.4 $\mu$mol/L), palmitic acid (125±44.8 $\mu$mol/L), linoleic acid (80.0±32.7 $\mu$mol/L), and stearic acid (60.5±16.8), contributing to 83.6% of total serum NEFAs at the baseline measurement (Table S2). Long-chain saturated FAs were more strongly correlated with long-chain monounsaturated FAs and trans FAs and correlated less strongly with n-6 and n-3 PUFAs (cutoff: 0.5; Figure S1). Additionally, long-chain monounsaturated FAs were strongly correlated with long-chain n-6 PUFAs and trans FAs and had moderate correlations with long-chain n-3 PUFAs.

Association of Serum Nonesterified FAs and CHD Composite

During the 11.7-year follow-up period, 434 cases of CHD composite, 266 cases of CHD death, and 271 cases of incident nonfatal MI occurred.

In the NEFA analysis, no significant associations were observed between NEFA subclasses (Table S3) or individual NEFAs (Table S4) and CHD composite in the fully adjusted model.

Association of Serum Nonesterified FAs and CHD Mortality

In NEFA subclass analysis, no significant associations were identified between NEFA subclasses and CHD mortality (Table S3).

Table S5 provides the results for 35 individual NEFAs. Table 2 is an excerpt from the table to highlight the key findings. In the multivariable fully adjusted Cox regression model nonesterified lauric acid (12:0) was associated with a 24% (95% CI, 2%–41%) lower risk of CHD mortality, whereas dihomo-$\gamma$-linoleic acid (20:3n-6) was associated with a 34% higher risk of CHD mortality (95% CI, 2%–76%) after adjusting for all covariates (Table 2).

Association of Serum Nonesterified FAs and Risk of Incident Nonfatal MI

No significant associations were identified between NEFA subclasses and incident nonfatal MI (Table S3).
Table 2. Significant Findings From Multivariable Adjusted Hazard Ratios Relating 35 Individual Serum NEFAs With Coronary Heart Disease Mortality and Incident Nonfatal Myocardial Infarction in the Cardiovascular Health Study Cohort With Baseline in 1996 to 1997

| NEFAs, µmol/L Per SD | CHD mortality | Nonfatal MI |
|----------------------|---------------|-------------|
|                      | Model 1*      | Model 2†    | Model 3‡    |                      |
|                       | HR (95% CI)   | P Value     | HR (95% CI) | P Value |
| Lauric acid, 12:0     | 0.75 (0.59–0.97) | 0.026 | 0.74 (0.57–0.96) | 0.024 | 0.76 (0.59–0.98) | 0.033 |
| Dihomo-γ-linolenic acid, 20:3n-6 | 1.40 (1.06–1.85) | 0.018 | 1.34 (1.02–1.76) | 0.037 | 1.34 (1.02–1.76) | 0.035 |
| Elaidic acid, 18:1n-9  | 1.40 (0.98–2.02) | 0.069 | 1.53 (1.06–2.22) | 0.025 | 1.46 (1.01–2.12) | 0.045 |

Values are hazard ratio (95% CI) per SD (n=1681). All 35 individual NEFAs were included in a single model in the Cox proportional hazard regression to estimate the risk of CHD mortality and incident nonfatal MI with 1 SD increment of each NEFA. CHD indicates coronary heart disease; HR, hazard ratio; MI, myocardial infarction; and NEFA, nonesterified fatty acid.

*Model 1 adjusted for age, sex, race, field center, education, and all 35 NEFAs.
†Model 2 adjusts for model 1 covariates plus smoking status, serum albumin, alcohol consumption, cystatin C for estimated glomerular filtration rate, weight, height, and physical activity.
‡Model 3 adjusts for Model 2 covariates plus hypertension and diabetes mellitus.

Table S6 provides the results for 35 individual NEFAs. Table 2 is an excerpt from the table to highlight the key findings. In the multivariable fully adjusted Cox regression model, elaidic acid (trans-18:1n-9, 18:1n-9) was associated with a 46% higher incident of nonfatal MI (95% CI, 1%–112%) per each SD increment (Table 2).

DISCUSSION

To our knowledge, this is the first study to profile subclasses and individual NEFAs and assess their relations with incident nonfatal MI and CHD mortality, in a community-based prospective study among older adults. Overall, no significant associations between subclasses of NEFA and incident nonfatal MI and CHD mortality were observed. In individual NEFA analysis, the findings were associated with 2 exploratory outcomes, and there were no significant associations between individual NEFAs and CHD composite. In a multivariable adjusted model, positive associations of nonesterified dihomo-γ-linolenic and elaidic acids with CHD mortality and incident nonfatal MI were identified, respectively. An inverse relation of medium-chain nonesterified lauric acid with CHD mortality was also observed. Although the concentrations of these individual NEFAs were relatively low in serum, the physiological effects may be of clinical importance. These findings extend the knowledge of previous work reporting positive associations of plasma total NEFAs with CHD risk.14,15,19

In the CHS participants, no NEFA subclasses or individual NEFAs were associated with CHD composite, and the associations for exploratory outcomes were inconsistent. A plausible explanation for this observation might be the differences of selection criteria between these 2 outcomes. Nonfatal MI was adjudicated by chest pain, cardiac enzymes, and ECG records30 and involves both type 1 (atherothrombosis) and type 2 MI (supply-demand mismatch from heterogeneity events) in this cohort.29,30 These were different pathophysiologically from fatal MI, which could lead to severe cardiac pump dysfunction, myocardial rupture, or arrhythmic death,53 and was considered as CHD death in this cohort. An alternate explanation relates to the FA reporting unit, absolute concentration versus percentage. Some prior work has suggested this variable may influence the final conclusions.34,35

Observed was a positive association between nonesterified dihomo-γ-linolenic acid and CHD mortality. This finding is consistent with prior reports from prospective studies that identified a positive association between plasma dihomo-γ-linolenic acid in both the PL and cholesteryl ester fractions and CHD risk.22,36 Also consistent with this finding, data from a nested case-control study of the WHI (Women's Health Initiative) Observational Study.37 WHI participants with CHD, compared with those without CHD, had lower estimated δ-5 desaturase activity, higher PL γ-linolenic acid, and dihomo-γ-linolenic acid concentrations.37 A plausible mechanism may be through hyperinsulinemia,38,39 a critical risk factor involved in the development of cardiovascular disease (CVD), including CHD. Dihomo-γ-linolenic acid is a precursor of arachidonic acid and can be synthesized endogenously from dietary linoleic acid, an essential FA, by a series of enzymic reactions including desaturation (δ-6 and δ-5 desaturases) and elongation (FA elongase 5). Hyperinsulinemia has been linked to increased concentration of PL dihomo-γ-linolenic acid and lower activity of δ-5 desaturase (fatty acid desaturase 1, FADS1).39,40 Although the causality and molecular mechanism remain unclear, genetic variation in FADS1 (TT genotype in rs174537) may play an important role in dihomo-γ-linolenic acid.
concentration\textsuperscript{41} and is associated with insulin resistance.\textsuperscript{40} Together, the observation in this and other studies suggests that nonesterified dihomo-\textgamma-linolenic acid may be a risk biomarker for CHD risk.

An inverse association was observed between nonesterified lauric acid (12:0) and CHD mortality. Prior work has identified a positive association between higher intake of lauric acid and incident MI and CHD.\textsuperscript{42,43} Lauric acid (12:0) is a medium-chain FA derived primarily from dairy fat and palm kernel oil\textsuperscript{44} or de novo lipogenesis. It is an intermediate for long-chain FA isomers synthesized by the liver.\textsuperscript{45} Because circulating nonesterified lauric acid in the fasting state is most likely synthesized endogenously,\textsuperscript{46} caution should be taken when interpreting these data.

In the CHS cohort a positive association was observed between serum nonesterified elaidic acid and incident nonfatal MI, but not with other nonesterified total or other trans FAs isomers. Previously, on the basis of a CHS nested case-control study, higher serum PL 18:2\textit{t} and lower 18:1\textit{f} were positively associated with fatal ischemic heart disease and sudden cardiac death.\textsuperscript{47} These data are consistent with some but not all prior work. The relation between serum PL and nonesterified total trans FAs and CVD, CHD, and CHD mortality\textsuperscript{20,48–52} appears to be dependent on whether the trans FA isomers were more likely to be derived from ruminant or partially hydrogenated fat.\textsuperscript{53,54} Regardless of the serum lipid fraction (PL, triacylglycerol or cholesteryl ester), 2 systematic reviews have reported a positive association between incident CHD and CVD risk factors and trans FA isomers of predominance in partially hydrogenated fat, particularly elaidic acid (18:1n-9f) and linoleic acid (18:2\textit{t}).\textsuperscript{53,55} An inverse association was also reported between incident CHD and CVD risk factors and trans FA isomers of predominance in ruminant fat, particularly trans-palmitoleic acid (16:1n-7t) and conjugated linoleic acid (18:2\textit{CLA}).\textsuperscript{53,55} Similar associations were observed in adipose tissues and MI risk.\textsuperscript{48,56–58} Discrepancies between the current and prior reports may be, in part, owing to the relative proportion of the ruminant or partially hydrogenated fat in the habitual diets of the cohorts, preferential incorporation into different lipid fractions, analytical ability to distinguish among the trans FA isomers, and/or covariates included in the analytical models. Food and Drug Administration legislation for mandatory inclusion of trans FA on Nutrition Facts labels and removal of partially hydrogenated fat from the Generally Recognized as Safe list has resulted in a drastic decline in the trans FA content of the food supply,\textsuperscript{59} and hence may have an impact on any observed nonsterified trans FA-CVD association. This also emphasized the importance of dietary source of trans FAs.

Strengths of this study are that the serum samples, incident nonfatal MI and CHD mortality data were collected in well-established research centers that also compiled extensive data on cardiometabolic risk factors, lifestyle, and demographics with little loss to follow-up. To account for the intercorrelation among individual NEFAs, adjusting for all 35 individual NEFAs in a single model allowed for the calculation of independent associations between individual NEFAs and incident nonfatal MI and CHD mortality. From an analytical perspective, coefficients of variation for individual NEFAs were low, particularly when some NEFA were present at low concentrations. A limitation was that the CHS participants were older adults, which limits the generalizability of the findings to other populations. Measures of the individual serum NEFAs were available at only 1 time point and not longitudinally, and thus serial measurements of individual NEFAs need to be considered in the future studies. Lastly, we were not aware of long-term studies that have assessed the long-term stability serum NEFA. There was a potential risk of PUFA oxidation before analysis, although the samples were stored at –80°C and never thawed.

CONCLUSIONS

In CHS participants, no significant association with CHD composite was identified. Both nonesterified elaidic and dihomo-\textgamma-linolenic acids were positively associated with risk of nonfatal MI and CHD mortality, respectively, suggesting potential susceptibility biomarkers for these 2 cardiovascular events.

ARTICLE INFORMATION

Received August 26, 2020; accepted February 3, 2021.

Affiliations

From the Cardiovascular Nutrition Laboratory, Jean Mayer USDA Human Nutrition Research Center on Aging, Tufts University, Boston, MA (N.K.H., N.R.M., A.H.L.); Department of Biostatistics, University of Washington, Seattle, WA (P.B.); Division of Aging, Brigham and Women’s Hospital, Harvard Medical School, Boston, MA (L.D.); Division of General Internal Medicine, University of California at Davis, CA (C.H.H.); Cardiology Section, San Francisco Veterans Affairs Health Care System, San Francisco, CA (J.R.K.); Department of Medicine, Epidemiology and Biostatistics, University of California San Francisco, San Francisco, CA (J.R.K.); Department of Neurology and Epidemiology, University of Washington, Seattle, WA (W.T.L.); and Division of General Medicine, Beth Israel Deaconess Medical Center, Boston, MA (K.J.M.).

Acknowledgments

We thank the participants and all the staff in the CHS Cohort.

Author contributions: Huang performed the data interpretation and wrote the initial draft of the article; Bůžková performed statistical analysis; Matthew and Lichtenstein oversaw the NEFA analysis and participated in data interpretation; Longstreth was a member of stroke adjudication committee and performed data interpretation; Hirsch and Kizer performed data interpretation; Djoussé and Mukamal designed the research and participated in data interpretation; Huang and Lichtenstein had primary responsibility for the final content of the article; all authors contributed to the critical review of the article and have approved the final version.
Serum NEFA and Incident Coronary Heart Disease

Sources of Funding
This study is supported by the National Institute on Aging, National Institutes of Health (R01AG053532, PFI Djoussé and Mukamal) and the U.S. Department of Agriculture (agreement no. 58-1950-4-401, PI Lichtenstein). The CHS was supported by contracts HHSN26820100036C, HHSN26820080007C, HHSN26820180001C, N01HC55222, N01HC55079, N01HC55080, N01HC55081, N01HC55082, N01HC55083, N01HC55086, and grants U01HL080295 and U01HL130114 from the National Heart, Lung, and Blood Institute, with additional contribution from the National Institute of Neurological Disorders and Stroke. Additional support was provided by R01AG023629 from the National Institute on Aging. 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 U.S. Department of Agriculture and the National Institutes of Health.

Disclosures
Kizer reports stock ownership in Bristol-Myers Squibb, Johnson & Johnson, Medtronic, Merck, and Pfizer. The remaining authors have no disclosures to report.

Supplementary Material
Tables S1–S6
Figure S1

REFERENCES
1. Virani SS, Alonso A, Benjamin EJ, Bittencourt MS, Callaway CW, Carson AP, Chamberlain AM, Chang AR, Cheng S, Delling FN, et al. Heart disease and stroke statistics-2020 update: a report from the American Heart Association. Circulation. 2020;141:e139–e596. DOI: 10.1161/CIR.0000000000000757.
2. Bentzon JF, Otsuka F, Virmani R, Falk E. Mechanisms of plaque formation and rupture. Circ Res. 2014;114:1852–1866. DOI: 10.1161/CIRCRESAHA.114.302721.
3. De Caterina R, Zampolli A, Del Turco S, Madonna R, Massaro M. Nutritional mechanisms that influence cardiovascular disease. Am J Clin Nutr. 2006;83:421S–426S. DOI: 10.1093/ajcn/83.2.421S.
4. Johnsson AR, Milner J, Makowski L. The inflammation highway: metabolism accelerates inflammatory traffic in obesity. Immunol Res. 2012;49:218–238. DOI: 10.1111/j.1600-065X.2012.01151.x.
5. Ly LD, Xu S, Choi SK, Ha CM, Thoudam T, Cha SK, Wiederkehr A, Hanley AJ. Association of NEFA composition with insulin sensitivity and relationship to dysglycemia. Am J Physiol Endocrinol Metab. 2019;318:e318–e329. DOI: 10.1152/ajpendo.00331.2019.
6. Delarue J, Magnan C. Free fatty acids and insulin resistance. Curr Opin Clin Nutr Metab Care. 2007;10:142–148. DOI: 10.1097/MCO.0b013e328024a9a0.
7. Sears B, Perry M. The role of fatty acids in insulin resistance. Lipids Health Dis. 2015;14:121. DOI: 10.1186/s12944-015-0123-1.
8. Ghosh A, Gao L, Thakur A, Siu PM, Lai CWK. Role of free fatty acids in endothelial dysfunction. J Biomed Sci. 2017;24:50. DOI: 10.1186/s12929-017-0357-5.
9. Florian JP, Paweleczyk JA. Non-esterified fatty acids increase arterial pressure via central sympathetic activation in humans. Clin Sci (Lond). 2009;116:61–69. DOI: 10.1042/CS20090063.
10. Fugmann M, Uhl O, Hellmuth C, Hetterich H, Malmberg K, Neal B, Nichols SJ, Rydén L, Meltlin L, et al. Adipoincin, free fatty acids, and cardiovascular outcomes in patients with type 2 diabetes and acute coronary syndrome. Diabetes Care. 2019;42:1792–1900. DOI: 10.2337/dc18-0158.
11. Djoussé L, Benkeser D, Arnold A, Kizer JR, Zieman SJ, Lemaire RN, Tracy RP, Gottdiener JS, Moazzarian D, Siscovick DS, et al. Plasma free fatty acids and risk of heart failure: the Cardiovascular Health Study. Circ Heart Fail. 2013;6:964–969. DOI: 10.1161/CIRCHEARTFAILURE.113.005021.
12. Khwaja O, Maziarz M, Biggs ML, Longstreth WT, Ix JH, Kizer JR, Zieman S, Tracy RP, Moazzarian D, Mukamal KJ, et al. Plasma free fatty acids and risk of stroke in the Cardiovascular Health Study. Int J Stroke. 2014;9:917–920. DOI: 10.1111/ijs.12216.
13. Djoussé L, Biggs ML, Ix JH, Kizer JR, Lemaire RN, Sotoodehnia N, Zieman SJ, Moazzarian D, Tracy RP, Mukamal KJ, et al. Nonesterified fatty acids and risk of sudden cardiac death in older adults. Circ Arhythms Electrophysiol. 2012;5:273–278. DOI: 10.1161/CIRCEP.111.967661.
14. He LY, Zhao JF, Han JL, Shen SS, Chen XJ. Correlation between serum free fatty acids levels and Ginsenni score in elderly patients with coronary heart disease. J Geriatr Cardiol. 2014;11:57–62.
15. Imamura F, Lemaire RN, King IB, Song X, Lichtenstein AH, Matthew NF, Herrington DM, Siscovick DS, Moazzarian D. Novel circulating fatty acid patterns and risk of cardiovascular disease: the Cardiovascular Health Study. Am J Clin Nutr. 2012;96:1252–1261. DOI: 10.3945/ajcn.120.039990.
16. Liu Q, Matthøn NR, Mansor JE, Howard BV, Tinker LF, Neuhauser ML, Van Horn LV, Rosouw JE, Allison MA, Martin LW, et al. Plasma phospholipid fatty acids and coronary heart disease risk: a matched case-control study within the Women’s Health Initiative Observational Study. Nutrients. 2019;11:1672. DOI: 10.3390/nu11071672.
17. Khaw KT, Friesen MD, Riboli E, Luben R, Wareham N. Plasma phospholipid fatty acid concentration and incident coronary heart disease in men and women: the Epic-Norfolk Prospective Study. PLoS Med. 2012;9(10):e1001255. DOI: 10.1371/journal.pmed.1001255.
18. Fried LP, Borhani NO, Enright P, Furberg CD, Gardin JM, Kronmal RA, Kuller LH, Manolio TA, Mettinger MB, Newman A, et al. the Cardiovascular Health Study: design and rationale. Ann Epidemiol. 1991;1:263–276. DOI: 10.1016/1047-2797(91)90005-W.
19. Matthøn NR, Ip B, Resteghini N, Ausman LM, Lichtenstein AH. Long-term fatty acid stability in human serum cholesterol ester, triglyceride, and phospholipid fractions. J Lipid Res. 2010;51:2626–2632. DOI: 10.1194/jlr.D007534.
20. Agren JJ, Jukunen A, Penttilä I. Rapid separation of serum lipids for fatty acid analysis by a single aminopropyl column. J Chromatogr. 2006;110:1871–1876. DOI: 10.1016/S0022-2875(02)04345-8.
21. Morrison WR, Smith LM. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride–methanol. J Lipid Res. 1964;5:600–608. DOI: 10.1194/jlr.D007534.
22. Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem. 1957;226:497–509. DOI: 10.1016/0021-9258(57)94489-5.
23. Matcalf LD, Schmitz AA, Pelka JR, McKinnon N, Zieman SJ, Tracy RP, Zieman S, Mukamal KJ, et al. Plasma free fatty acids and cardiovascular outcomes in patients with type 2 diabetes and acute coronary syndrome: the Epic- Norfolk Prospective Study. Ann Epidemiol. 2013;23:90–95. DOI: 10.1016/j.annepidem.2012.06.006.
24. Taylor HL, Jacobs DR Jr, Schucker B, Knudsen J, Leon AS, Debacker G, Theroux S. Surveillance and ascertainment of cardiovascular events, the Cardiovascular Health Study. Ann Epidemiol. 1993;3:504–507. DOI: 10.1016/1047-2797(93)90105-D.
25. Cheung CM, Wang CC, Lui JT, Liu SC, Chang YL, Wang CC, Chan SF, et al. Association of NEFA composition with insulin sensitivity and beta cell function in the Prospective Metabolism and Islet Cell Evaluation (PROMISE) cohort. Diabetologia. 2016;61:621–630. DOI: 10.1007/s00125-017-4534-6.
26. Huang et al Serum NEFA and Incident Coronary Heart Disease. 2018;61:621–630. DOI: 10.1007/s00125-017-4534-6.
a pooled analysis of 3,418 individuals with CKD. Am J Kidney Dis. 2008;51:395–406. DOI: 10.1053/j.ajkd.2007.11.018.

33. Gupta S, Vaidya SR, Arora S, Bahekar A, Devarapalli SR. Type 2 versus type 1 myocardial infarction: a comparison of clinical characteristics and outcomes with a meta-analysis of observational studies. Cardiovasc Diagn Ther. 2017;7:348–358. DOI: 10.21037/cd.t.2017.03.21.

34. Sergeant S, Rucinski I, Ivester P, Lee TC, Morgan TM, Nicklas BJ, Mathias RA, Chilton FH. Impact of methods used to express levels of circulating fatty acids on the degree and direction of associations with blood lipids in humans. Br J Nutr. 2016;115:251–261. DOI: 10.1017/S0007114515004341.

35. Wanders AJ, Alssema M, van Greevenbroek MJ, Elshorbagy A, Zock PL, Dekker JM. Plasma phospholipid polyunsaturated fatty acid composition of dietary fat quality and endogenous metabolism predict coronary heart disease risk: a nested case-control study within the Women’s Health Initiative Observational Study. J Am Heart Assoc. 2014;3:e000764. DOI: 10.1161/JAHA.113.000764.

36. Wang L, Folsom AR, Eckfeldt JH. Plasma fatty acid composition and incidence of coronary heart disease in middle aged adults: the Atherosclerosis Risk in Communities (ARIC) study. Nutr Metab Cardiovasc Dis. 2003;13:256–266. DOI: 10.1016/S0939-4753(03)80029-7.

37. Matthys NW, Oei EM, Van Horn L, Neuhausser ML, Woodman R, Lichtenstein AH. Plasma phospholipid fatty acid biomarkers of dietary fat quality and endogenous metabolism predict coronary heart disease risk: a nested case-control study within the Women’s Health Initiative Observational Study. J Am Heart Assoc. 2014;3:e000764. DOI: 10.1161/JAHA.113.000764.

38. Tauraz Y, Inoue K, Sugisawa C, Saito J, Omura M, Nishikawa T. Increased serum d(homo)-γ-linolenic acid levels are associated with obesity, body fat accumulation, and insulin resistance in Japanese patients with Type 2 diabetes. Intern Med. 2018;57:2929–2935. DOI: 10.2169/internalmedicine.0816-18.

39. Weir NL, Nomura SO, Steffen BT, Guan W, Karger AB, Klein R, Klein BEK, Cotch MF, Tsai MY. Associations between omega-6 polyunsaturated fatty acids, hyperinsulinemia and incident diabetes by race/ethnicity: the Multi-Ethnic Study of Atherosclerosis. Clin Nutr. 2020;39:3031–3041. DOI: 10.1016/j.clnu.2020.01.003.

40. Kim OY, Lim HH, Yang LI, Chae JS, Lee JH. Fatty acid desaturase (FADS) gene polymorphisms and insulin resistance in association with serum phospholipid polyunsaturated fatty acid composition in healthy Korean men; cross-sectional study. Nutr Metab (Lond). 2011;6:24. DOI: 10.1186/1743-7075-8-24.

41. Sergeant S, Hallmark B, Mathias RA,必须TL, Ivester P, Bohannon ML, Rucinski I, Johnstone L, Seeds MC, Chilton FH. Prospective clinical trial examining the impact of genetic variation in FADS1 on the metabolism of linoleic acid–containing botanical oils. J Am Clin Nutr. 2020;111:1068–1078. DOI: 10.1093/ajcn/nqaa023.

42. Kabagambe EK, Baylin A, Siles X, Campos H. Individual saturated fatty acids and nonfatal acute myocardial infarction in Costa Rica. Eur J Clin Nutr. 2003;57:1447–1457. DOI: 10.1038/sj.ejcn.1601709.

43. Zong G, Li Y, Wanders AJ, Alssema M, Zock PL, Willett WC, Hu FB, Sun Q. Intake of individual saturated fatty acids and risk of coronary heart disease in US men and women: two prospective longitudinal cohort studies. BMJ. 2016;355:i5796. DOI: 10.1136/bmj.i5796.

44. US Department of Agriculture, Agricultural Research Service. Nutrient Data Laboratory. USDA National Nutrient Database for Standard Reference, Release 28 (Slightly revised). Version Current: May 2016. Available at: http://www.ars.usda.gov/nea/bhnrc/masf/. Accessed July 30, 2020.

45. McCarty MF, DiNicolantonio JJ. Lactic acid-rich medium-chain triacylglycerides can substitute for other oils in cooking applications and may have limited pathogenicity. Open Heart. 2016;3:e000467. DOI: 10.1136/openhrt-2016-000467.

46. Schönfeld P, Wojtczak L. Short- and medium-chain fatty acids in energy metabolism: the cellular perspective. J Lipid Res. 2016;57:943–954. DOI: 10.1194/jr.P067629.

47. Lemaitre RN, King IB, Mozaffarian D, Sotoodehnia N, Rea TD, Kuller LH, Tracy RP, Siscovick DS. Plasma phospholipid trans fatty acids, fatal ischemic heart disease, and sudden cardiac death in older adults: the Cardiovascular Health Study. Circulation. 2006;114:209–215.

48. de Souza RJ, Mente A, Moarellou A, Cozma AI, Ha V, Kishibe T, Uleryk E, Budyalowski P, Schühnem H, Beyene J, et al. Intake of saturated and trans unsaturated fatty acids and risk of all-cause mortality, cardiovascular disease, and type 2 diabetes: systematic review and meta-analysis of observational studies. BMJ. 2015;351:h3978. DOI: 10.1136/bmj.h3978.

49. Brandt EJ, Myerson R, Perrallion MC, Polonsky TS. Hospital admissions for myocardial infarction and stroke before and after the trans fatty acid restrictions in New York. JAMA Cardiol. 2017;2:627–634. DOI: 10.1001/jamacardio.2017.0491.

50. Mozaffarian D, Katan MB, Ascherio A, Stampfer MJ, Willett WC. Trans fatty acids and cardiovascular disease. N Engl J Med. 2006;354:1601–1613. DOI: 10.1056/NEJMa054035.

51. Restrepo BJ. Further decline of trans fatty acids levels among US adults between 1999–2000 and 2009–2010. Am J Public Health. 2017;107:156–158. DOI: 10.2105/AJPH.2016.303524.

52. Li H, Zhang Q, Song J, Wang A, Zou Y, Ding L, Wen Y. Plasma trans fatty acids levels and mortality: a cohort study based on 1999–2000 National Health and Nutrition Examination Survey (NHANES). Lipids Health Dis. 2016;15:176. DOI: 10.1186/s12944-017-0567-6.

53. Gayet-Boyer C, Tenenhaus-Aziza F, Prunet C, Marmonier C, Malpuech-Brugere C, Lamarche B, Chardigny JM. Is there a linear relationship between the dose of ruminant trans-fatty acids and cardiovascular risk markers in healthy subjects: results from a systematic review and meta-regression of randomised clinical trials. Br J Nutr. 2014;112:1914–1922.

54. Micha R, King IB, Lemaitre RN, Rimm EB, Sacks F, Song X, Siscovick DS, Mozaffarian D. Food sources of individual plasma phospholipid trans fatty acid isomers: the Cardiovascular Health Study. Am J Clin Nutr. 2010;91:883–893. DOI: 10.3945/ajcn.2009.28877.

55. Bendsen NT, Christensen R, Bartels EM, Astrup A. Consumption of industrial and ruminant trans fatty acids and risk of coronary heart disease: a systematic review and meta-analysis of cohort studies. Eur J Clin Nutr. 2011;65:773–783. DOI: 10.1038/ejcn.2011.34.

56. Luan D, Wang D, Campos H, Baylin A. Adipose tissue palmitoleic acid is inversely associated with nonfatal acute myocardial infarction in Costa Rican adults. Nutr Metab Cardiovasc Dis. 2018;28:973–979. DOI: 10.1016/j.numecd.2018.05.004.

57. Smit LA, Baylin A, Campos H. Conjugated linoleic acid in adipose tissue and risk of myocardial infarction. Am J Clin Nutr. 2010;92:34–40. DOI: 10.3945/ajcn.2010.29524.

58. Jakobsen MU, Gorst-Rasmussen A, Eriksen HH, Stegger J, Joensen AM, Tjønneland A, Dyerberg J, Schmidt EB, Overvad K. Polyunsaturated fatty acid isomers for myocardial infarction and stroke before and after the trans fatty acid restrictions in New York. JAMA Cardiol. 2017;2:627–634. DOI: 10.1001/jamacardio.2017.0491.
Supplemental Material
Table S1. Baseline characteristics of 1681 participants in the Cardiovascular Health Study cohort at baseline in 1996-1997.

| Characteristics                | Non-included Participants (N=2732) | Included Participants (N=1681) | P-value |
|-------------------------------|------------------------------------|-------------------------------|---------|
| Age, year                     | 79.2 ± 5.43                        | 77.6 ± 4.44                   | <0.001  |
| Male, %                       | 39.9                               | 35.8                          | 0.006   |
| African-American, %           | 18.1                               | 14.5                          | 0.002   |
| CHS clinic, %                 |                                    |                               | 0.001   |
| California                    | 24.8                               | 29.0                          |         |
| Maryland                      | 21.7                               | 20.5                          |         |
| North Carolina                | 27.6                               | 23.4                          |         |
| Pennsylvania                  | 25.8                               | 27.1                          |         |
| Educational attainment, %     |                                    |                               | <0.001  |
| ≥High school                  | 41.3                               | 50.8                          |         |
| Smoking status, %             |                                    |                               | <0.001  |
| Never smoked                  | 45.2                               | 51.4                          |         |
| Former smoker                 | 44.9                               | 40.3                          |         |
| Current smoker                | 9.9                                | 8.3                           |         |
| Alcoholic drinks/week, %      |                                    |                               | <0.001  |
| 0                             | 63.5                               | 55.0                          |         |
| 1-6                           | 26.9                               | 30.7                          |         |
| 7-14                          | 5.6                                | 8.4                           |         |
| >14                           | 3.9                                | 5.9                           |         |
| Hypertension, %               | 69.5                               | 59.2                          | <0.001  |
| Diabetes, %                   | 30.9                               | 2.4                           | <0.001  |
| Prevalent AF*                 | 6.4                                | 3.3                           | <0.001  |
| Prevalent CHF†                | 15.9                               | 2.9                           | <0.001  |
| Prevalent Stroke, %           | 10.9                               | 3.6                           | <0.001  |
|                                | Mean ± SD      | Mean ± SD      | p-value |
|--------------------------------|----------------|----------------|---------|
| Prevalent TIA ‡, %             | 5.4            | 2.7            | <0.001  |
| Hypertension medication, %     | 67.1           | 46.5           | <0.001  |
| Estrogen, %                    | 12.7           | 19.5           | <0.001  |
| Fasting glucose, mg/dL         | 115.4 ± 41.3   | 97.7 ± 14.5    | <0.001  |
| Albumin, g/dL                  | 3.84 ± 0.31    | 3.82 ± 0.29    | 0.027   |
| Body mass index, kg/m²         | 27.1 ± 4.8     | 26.7 ± 4.4     | 0.034   |
| eGFRcysc §                     | 67.7 ± 20.1    | 73.2 ± 18.5    | <0.001  |
| C-Reactive Protein, mg/dL, log²| 1.44 ± 1.59    | 1.20 ± 1.58    | <0.001  |
| Waist circumference, cm        | 98.0 ± 13.6    | 96.2 ± 12.9    | <0.001  |

Values are presented as mean±SD for continuous variables and percent for categorical variables.

*AF, atrial fibrillation; †CHF, congestive heart failure; ‡TIA, transient ischemic attack; §eGFRcysc, cystatin C for estimate glomerular filtration rate.
Table S2. Mean and standard deviation (S.D.) for individual non-esterified fatty acids in the Cardiovascular Health Study participants at baseline in 1996-1997.

| NEFA, µmol/L             | Mean ± SD | Interquartile Range |
|---------------------------|-----------|---------------------|
| SFA                       | 201.1 ± 62.8 | 78.6               |
| Lauric acid, 12:0         | 2.72 ± 2.81  | 1.72               |
| Myristic acid, 14:0       | 9.13 ± 4.10  | 5.01               |
| Pentadecylic acid, 15:0   | 1.63 ± 0.54  | 0.66               |
| Palmitic acid, 16:0       | 125.3 ± 44.8 | 55.1               |
| Stearic acid, 18:0        | 60.5 ± 16.8  | 20.3               |
| Arachidic acid, 20:0      | 0.72 ± 0.37  | 0.31               |
| Behenic acid, 22:0        | 0.43 ± 0.18  | 0.13               |
| Lignoceric acid, 24:0     | 0.66 ± 0.62  | 0.24               |
| MUFA                      | 186.2 ± 80.1 | 105                |
| Myristoleic acid, 14:1n-5 | 0.90 ± 0.65  | 0.74               |
| 7-hexadecenoic acid, 16:1n-9 | 2.02 ± 0.86  | 1.08               |
| Palmitoleic acid, 16:1n-7 | 17.0 ± 11.4  | 12.6               |
| Oleic acid, 18:1n-9       | 152.9 ± 63.4 | 83.0               |
| 7-Vaccenic acid, 18:1n-7  | 11.6 ± 5.52  | 7.02               |
| Gondoic acid, 20:1n-9     | 1.05 ± 0.48  | 0.59               |
| Erucic acid, 22:1n-9      | 0.38 ± 0.22  | 0.21               |
| Nervonic acid, 24:1n-9    | 0.35 ± 0.19  | 0.11               |
| n-6 PUFA                  | 88.9 ± 35.2  | 46.6               |
| Fatty Acid                          | %  | mg/g |
|------------------------------------|----|------|
| Linoleic acid, 18:2n-6             | 80.0 ± 32.7 | 43.4 |
| γ-Linolenic acid, 18:3n-6          | 0.56 ± 0.32 | 0.37 |
| Dihomolinoleic acid, 20:2n-6       | 0.90 ± 0.42 | 0.50 |
| Dihomo-γ-Linolenic acid, 20:3n-6   | 0.96 ± 0.69 | 0.54 |
| Arachidonic acid, 20:4n-6          | 5.38 ± 2.93 | 2.79 |
| Adrenic acid, 22:4n-6              | 0.72 ± 0.48 | 0.43 |
| Docosapentaenoic acid, 22:5n-6     | 0.39 ± 0.21 | 0.21 |
| **n-3 PUFA**                       |    |      |
| Alpha Linolenic acid (ALA), 18:3n-3 | 5.85 ± 2.94 | 3.58 |
| Stearidonic acid (SDA), 18:4n-3    | 2.15 ± 1.05 | 1.23 |
| Eicosapentaenoic acid (EPA), 20:5n-3 | 0.37 ± 0.29 | 0.27 |
| Docosapentaenoic acid (DPA), 22:5n-3 | 0.86 ± 0.44 | 0.50 |
| Docosahexaenoic acid (DHA), 22:6n-3 | 2.44 ± 1.48 | 1.43 |
| **trans fatty acid**               |    |      |
| *trans*-7-hexadecenoic acid, 16:1n-9t | 0.89 ± 0.48 | 0.57 |
| Palmitelaidic acid, 16:1n-7t       | 0.88 ± 0.36 | 0.45 |
| Petroselinic acid, 18:1n-10-12t    | 0.71 ± 0.37 | 0.42 |
| Elaidic acid, 18:1n-9              | 6.58 ± 2.90 | 3.72 |
| *trans*-Vaccenic acid, 18:1n-7t    | 2.75 ± 1.19 | 1.54 |
| Linoelaidic acid, 18:2t†           | 0.23 ± 0.20 | 0.20 |
| Conjugated linoleic acid, 18:2CLA  | 1.07 ± 0.76 | 0.91 |
Values are mean ± standard deviation (n=1,681). SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; *18:1n10-12t, sum of 18:2n-10, n-11, and n-12 trans isomers; †18:2t, sum of all 18:2 trans isomers.
Table S3. Multivariable adjusted hazard ratio according to sub-classes of serum NEFA with CHD composite, coronary heart disease (CHD) mortality and incident non-fatal myocardial infarction (MI) in the Cardiovascular Health Study cohort at baseline in 1996-1997.

| NEFA sub-classes, per SD | Model 1\(^*\) | Model 2\(^+\) | Model 3\(^\dagger\) |
|-------------------------|----------------|----------------|---------------------|
|                         | Hazard ratio (95% CI) | P-value | Hazard ratio (95% CI) | P-value | Hazard ratio (95% CI) | P-value |
| **SFA**                 |                 |                 |                     |                     |                     |
| CHD composite           | 0.94 (0.77-1.16) | 0.586          | 0.97 (0.78-1.20)    | 0.761          | 0.95 (0.77-1.78)    | 0.628          |
| CHD mortality           | 0.91 (0.69-1.19) | 0.478          | 0.94 (0.71-1.24)    | 0.653          | 0.93 (0.70-1.22)    | 0.579          |
| Non-fatal MI            | 1.01 (0.78-1.31) | 0.927          | 1.05 (0.81-1.36)    | 0.737          | 1.03 (0.80-1.34)    | 0.807          |
| **MUFA**                |                 |                 |                     |                     |                     |
| CHD composite           | 0.96 (0.74-1.24) | 0.730          | 0.91 (0.70-1.19)    | 0.479          | 0.92 (0.71-1.21)    | 0.555          |
| CHD mortality           | 1.21 (0.87-1.68) | 0.268          | 1.13 (0.80-1.58)    | 0.491          | 1.13 (0.81-1.59)    | 0.467          |
| Non-fatal MI            | 0.79 (0.57-1.09) | 0.153          | 0.76 (0.55-1.07)    | 0.115          | 0.77 (0.55-1.08)    | 0.134          |
| **n-6 PUFA**            |                 |                 |                     |                     |                     |
| CHD composite           | 1.03 (0.80-1.32) | 0.837          | 1.04 (0.80-1.36)    | 0.762          | 1.04 (0.80-1.35)    | 0.767          |
| CHD mortality           | 0.83 (0.60-1.15) | 0.256          | 0.88 (0.63-1.23)    | 0.437          | 0.87 (0.62-1.21)    | 0.406          |
| Non-fatal MI            | 1.18 (0.86-1.62) | 0.296          | 1.16 (0.84-1.61)    | 0.370          | 1.16 (0.84-1.61)    | 0.377          |
| **n-3 PUFA**            |                 |                 |                     |                     |                     |
| CHD composite           | 0.91 (0.74-1.01) | 0.342          | 0.95 (0.78-1.17)    | 0.643          | 0.95 (0.78-1.17)    | 0.650          |
|                          | CHD mortality | Non-fatal MI |
|--------------------------|---------------|--------------|
|                          | 0.93 (0.71-1.20) | 0.96 (0.75-1.22) |
| 0.563                    | 0.726         |
| 0.95 (0.73-1.23)         | 1.03 (0.80-1.32) |
| 0.674                    | 0.818         |
| 0.96 (0.74-1.25)         | 1.02 (0.80-1.31) |
| 0.762                    | 0.872         |

**trans fatty acid**

|                          | CHD composite | CHD mortality | Non-fatal MI |
|--------------------------|---------------|---------------|--------------|
|                          | 1.18 (1.02-1.36) | 1.26 (1.05-1.50) | 1.15 (0.96-1.38) |
| 0.023                    | 0.012         | 0.122         |
| 1.13 (0.97-1.31)         | 1.18 (0.98-1.42) |
| 0.115                    | 0.077         |
| 1.13 (0.98-1.32)         | 1.20 (0.99-1.44) |
| 0.103                    | 0.059         |
|                          |               |               |              |

Values are hazard ratio (95% confidence interval) per standard deviation ($n=1,681$). CHD composite includes CHD mortality and non-fatal MI. CI, confidence interval; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid. *Model 1 adjusted for age, sex, race, field center, education, and all other NEFA sub-classes; †Model 2 adjusts for model 1 covariates plus smoking status, serum albumin, alcohol consumption, cystatin C for estimate glomerular filtration rate, weight, height, and physical activity; ‡Model 3 adjusts for Model 2 covariates plus hypertension and diabetes.
Table S4. Multivariable adjusted hazard ratios relating 35 individual serum NEFAs with coronary heart disease (CHD) composite in the Cardiovascular Health Study cohort at baseline in 1996-1997.

| NEFAs, µmol/L per SD | Model 1* | | Model 2† | | Model 3‡ |
|---------------------|---------|----------------|---------|----------------|---------|
|                     | HR (95% CI) | P-value | HR (95% CI) | P-value | HR (95% CI) | P-value |
| SFA                 |         |         |         |         |         |         |
| Lauric acid, 12:0   | 0.97    | (0.86, 1.10) | 0.633 | 0.96    | (0.85, 1.10) | 0.567 | 0.97    | (0.85, 1.10) | 0.617 |
| Myristic acid, 14:0 | 1.27    | (0.87, 1.85) | 0.222 | 1.18    | (0.81, 1.72) | 0.387 | 1.15    | (0.79, 1.67) | 0.461 |
| Pentadecylic acid, 15:0 | 0.77  | (0.57, 1.04) | 0.091 | 0.80    | (0.59, 1.08) | 0.141 | 0.80    | (0.59, 1.09) | 0.153 |
| Palmitic acid, 16:0 | 1.14    | (0.79, 1.64) | 0.494 | 1.08    | (0.75, 1.57) | 0.678 | 1.04    | (0.72, 1.51) | 0.822 |
| Stearic acid, 18:0  | 0.91    | (0.69, 1.18) | 0.466 | 0.95    | (0.72, 1.24) | 0.680 | 0.95    | (0.72, 1.24) | 0.701 |
| Arachidic acid, 20:0| 1.01    | (0.84, 1.22) | 0.927 | 1.04    | (0.85, 1.26) | 0.725 | 1.04    | (0.86, 1.27) | 0.668 |
| Behenic acid, 22:0  | 0.98    | (0.86, 1.11) | 0.735 | 0.97    | (0.85, 1.12) | 0.694 | 0.96    | (0.84, 1.11) | 0.591 |
| Lignoceric acid, 24:0| 0.99  | (0.91, 1.08) | 0.862 | 1.00    | (0.91, 1.09) | 0.919 | 1.00    | (0.92, 1.08) | 0.920 |
| MUFA                |         |         |         |         |         |         |
| Myristoleic acid, 14:1 n-5 | 0.95  | (0.67, 1.33) | 0.748 | 0.93    | (0.67, 1.31) | 0.688 | 0.95    | (0.68, 1.33) | 0.760 |
| cis-7-hexadecenoic acid, 16:1 n-9 | 1.03 | (0.75, 1.43) | 0.842 | 1.02    | (0.74, 1.41) | 0.909 | 1.04    | (0.75, 1.45) | 0.804 |
| Palmitoleic acid, 16:1 n-7 | 0.77 | (0.50, 1.20) | 0.252 | 0.87    | (0.56, 1.37) | 0.558 | 0.92    | (0.59, 1.44) | 0.717 |
| Oleic acid, 18:1 n-9 | 0.56    | (0.30, 1.03) | 0.063 | 0.61    | (0.33, 1.12) | 0.109 | 0.61    | (0.33, 1.12) | 0.110 |
|                |       |       |       |       |
|----------------|-------|-------|-------|-------|
| cis-Vaccenic acid, 18:1 n-7 | 1.57  | 0.060 | 1.37  | 0.203 |
|                | (0.98, 2.51) | (0.85, 2.20) | (0.79, 2.08) | (0.80, 2.08) |
| Gondoic acid, 20:1 n-9 | 1.04  | 0.783 | 1.03  | 0.834 |
|                | (0.77, 1.41) | (0.77, 1.37) | (0.79, 1.41) | (0.79, 1.41) |
| Erucic acid, 22:1 n-9 | 1.03  | 0.587 | 1.03  | 0.585 |
|                | (0.92, 1.16) | (0.92, 1.16) | (0.91, 1.15) | (0.91, 1.15) |
| Nervonic acid, 24:1 n-9 | 1.08  | 0.059 | 1.09  | 0.051 |
|                | (1.00, 1.18) | (1.00, 1.18) | (0.99, 1.18) | (0.99, 1.18) |
| **n-6 PUFA** |       |       |       |       |
| Linoleic acid, 18:2 n-6 | 1.29  | 0.214 | 1.18  | 0.422 |
|                | (0.87, 1.92) | (0.79, 1.78) | (0.78, 1.79) | (0.78, 1.79) |
| γ-Linolenic acid, 18:3 n-6 | 0.96  | 0.572 | 0.99  | 0.879 |
|                | (0.83, 1.11) | (0.86, 1.14) | (0.85, 1.14) | (0.85, 1.14) |
| Dihomolinoelic acid, 20:2 n-6 | 0.94  | 0.525 | 0.99  | 0.942 |
|                | (0.76, 1.15) | (0.81, 1.22) | (0.79, 1.24) | (0.79, 1.24) |
| Dihomo-γ-linolenic acid, 20:3 n-6 | 1.13  | 0.286 | 1.10  | 0.380 |
|                | (0.90, 1.41) | (0.89, 1.37) | (0.89, 1.37) | (0.89, 1.37) |
| Arachidonic acid, 20:4 n-6 | 0.88  | 0.177 | 0.86  | 0.124 |
|                | (0.73, 1.06) | (0.71, 1.04) | (0.72, 1.07) | (0.72, 1.07) |
| Adrenic acid, 22:4 n-6 | 1.00  | 0.947 | 0.97  | 0.622 |
|                | (0.88, 1.12) | (0.85, 1.10) | (0.83, 1.09) | (0.83, 1.09) |
| Docosapentaenoic acid, 22:5n-6 | 0.99  | 0.859 | 1.04  | 0.641 |
|                | (0.83, 1.17) | (0.88, 1.23) | (0.88, 1.24) | (0.88, 1.24) |
| **n-3 PUFA** |       |       |       |       |
| α-linolenic acid, 18:3n-3 | 0.80  | 0.107 | 0.88  | 0.341 |
|                | (0.62, 1.05) | (0.67, 1.51) | (0.67, 1.15) | (0.67, 1.15) |
| Stearidonic acid, 18:4n-3 | 0.97  | 0.681 | 0.94  | 0.360 |
|                | (0.86, 1.10) | (0.83, 1.07) | (0.85, 1.10) | (0.85, 1.10) |
| Eicosapentaenoic acid, 20:5n-3 | 1.12  | 0.171 | 1.11  | 0.228 |
|                | (0.95, 1.33) | (0.94, 1.31) | (0.92, 1.29) | (0.92, 1.29) |
| Docosapentaenoic acid, 22:5n-3 | 1.12  | 0.399 | 1.15  | 0.296 |
|                | (0.86, 1.44) | (0.88, 1.50) | (0.88, 1.49) | (0.88, 1.49) |
| Docosahexaenoic acid, 22:6n-3 | 0.80  | 0.042 | 0.81  | 0.058 |
|                | (0.65, 0.99) | (0.66, 1.01) | (0.66, 1.02) | (0.66, 1.02) |

**trans fatty acid**
| NEFA                        | Hazard Ratio | 95% CI         | Hazard Ratio | 95% CI         | Hazard Ratio | 95% CI         |
|-----------------------------|--------------|----------------|--------------|----------------|--------------|----------------|
| trans-7-hexadecenoic acid, 16:1n-9t | 0.97         | (0.72, 1.41)   | 0.95         | (0.70, 1.30)   | 0.741        | (0.70, 1.31)   |
| Palmitelaidic acid, 16:1n-7t | 1.21         | (0.90, 1.63)   | 1.17         | (0.87, 1.58)   | 0.309        | (0.88, 1.60)   |
| Petroselinic acid, 18:1n-10-12t| 1.09         | (0.78, 1.54)   | 1.02         | (0.72, 1.44)   | 0.920        | (0.73, 1.45)   |
| Elaidic acid, 18:1n-9t      | 1.14         | (0.85, 1.52)   | 1.23         | (0.92, 1.65)   | 0.163        | (0.89, 1.60)   |
| trans-Vaccenic acid, 18:1n-7t| 0.89         | (0.68, 1.18)   | 0.88         | (0.67, 1.17)   | 0.380        | (0.69, 1.20)   |
| Linoelaidic acid, 18:2t| 0.98         | (0.88, 1.09)   | 1.02         | (0.91, 1.13)   | 0.778        | (0.92, 1.14)   |
| Conjugated linoleic acid, 18:2CLA| 1.07         | (0.92, 1.25)   | 1.06         | (0.91, 1.24)   | 0.450        | (0.89, 1.21)   |

Values are hazard ratio (95% confidence interval) per standard deviation (n=1,681). CHD composite includes CHD mortality and non-fatal MI. All 35 individual NEFAs were included in a single model in the Cox proportional hazard regression to estimate the risk of CHD composite with one standard deviation increment of each NEFA. CI, confidence interval; HR, hazard ratio; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SD, standard deviation; SFA, saturated fatty acid. *Model 1 adjusted for age, sex, race, field center, education, and all other 35 NEFAs; †Model 2 adjusts for model 1 covariates plus smoking status, serum albumin, alcohol consumption, cystatin C for estimate glomerular filtration rate, weight, height, and physical activity; ‡Model 3 adjusts for Model 2 covariates plus hypertension and diabetes; §18:1n-10-12t, sum of 18:2n-10, n-11, and n-12 trans isomers; ‖18:2t, sum of all 18:2 trans isomers; #18:2CLA, conjugated linoleic acid.
Table S5. Multivariable adjusted hazard ratios relating 35 individual serum NEFAs with coronary heart disease (CHD) mortality in the Cardiovascular Health Study cohort at baseline in 1996-1997

| NEFAs, µmol/L per SD | Model 1<sup>*</sup> | Model 2<sup>†</sup> | Model 3<sup>‡</sup> |
|----------------------|--------------------|--------------------|--------------------|
|                      | HR (95% CI)        | P-value            | HR (95% CI)        | P-value            | HR (95% CI)        | P-value            |
| **SFA**              |                    |                    |                    |                    |                    |                    |
| Lauric acid, 12:0    | 0.75 (0.59, 0.97)  | 0.026              | 0.74 (0.57, 0.96)  | 0.024              | 0.76 (0.59, 0.98)  | 0.033              |
| Myristic acid, 14:0  | 1.42 (0.87, 2.32)  | 0.162              | 1.29 (0.79, 2.11)  | 0.302              | 1.26 (0.78, 2.04)  | 0.351              |
| Pentadecylic acid, 15:0 | 0.79 (0.54, 1.15) | 0.212              | 0.84 (0.57, 1.23)  | 0.361              | 0.84 (0.57, 1.23)  | 0.364              |
| Palmitic acid, 16:0  | 1.00 (0.64, 1.58)  | 0.996              | 0.98 (0.62, 1.54)  | 0.925              | 0.97 (0.61, 1.52)  | 0.876              |
| Stearic acid, 18:0   | 0.92 (0.66, 1.29)  | 0.619              | 0.95 (0.68, 1.34)  | 0.774              | 0.94 (0.67, 1.33)  | 0.730              |
| Arachidic acid, 20:0 | 1.09 (0.86, 1.37)  | 0.475              | 1.09 (0.86, 1.38)  | 0.489              | 1.10 (0.87, 1.40)  | 0.426              |
| Behenic acid, 22:0   | 0.99 (0.85, 1.17)  | 0.931              | 1.00 (0.84, 1.18)  | 0.974              | 0.99 (0.83, 1.17)  | 0.883              |
| Lignoceric acid, 24:0| 0.98 (0.83, 1.15)  | 0.771              | 0.99 (0.85, 1.15)  | 0.861              | 0.99 (0.85, 1.15)  | 0.874              |
| **MUFA**             |                    |                    |                    |                    |                    |                    |
| Myristoleic acid, 14:1 n-5 | 0.98 (0.64, 1.50) | 0.932              | 0.99 (0.65, 1.49)  | 0.952              | 1.01 (0.67, 1.52)  | 0.960              |
| cis-7-hexadecenoic acid, 16:1 n-9 | 0.87 (0.57, 1.34) | 0.526              | 0.84 (0.54, 1.30)  | 0.427              | 0.84 (0.54, 1.31)  | 0.441              |
| Palmitoleic acid, 16:1 n-7 | 0.87 (0.51, 1.49) | 0.607              | 0.96 (0.56, 1.68)  | 0.897              | 1.00 (0.58, 1.73)  | 0.997              |
| Oleic acid, 18:1 n-9  | 0.63 (0.29, 1.39)  | 0.249              | 0.71 (0.33, 1.52)  | 0.374              | 0.72 (0.33, 1.56)  | 0.399              |
\begin{table}[h]
\centering
\begin{tabular}{lcccccc}
\hline
 & cis-Vaccenic acid, & & & & & \\
 & 18:1 n-7 & 1.67 & 1.43 & 0.232 & 1.34 & 0.322 \\
 & & (0.94, 2.96) & (0.80, 2.55) & & (0.75, 2.42) & \\
Gondoic acid, 20:1 & & & & & & \\
n-9 & 1.18 & 1.16 & 0.400 & 1.17 & 0.368 \\
 & & (0.82, 1.71) & (0.82, 1.63) & & (0.83, 1.66) & \\
Erucic acid, 22:1 n-9 & & & & & & \\
 & 1.02 & 1.01 & 0.893 & 1.01 & 0.908 \\
 & & (0.88, 1.19) & (0.87, 1.18) & & (0.86, 1.18) & \\
Nervonic acid, 24:1 & & & & & & \\
n-9 & 1.09 & 1.10 & 0.049 & 1.10 & 0.062 \\
 & & (0.99, 1.21) & (1.00, 1.21) & & (1.00, 1.21) & \\
n-6 PUFA & & & & & & \\
Linoleic acid, 18:2 & & & & & & \\
n-6 & 0.79 & 0.73 & 0.239 & 0.72 & 0.215 \\
 & & (0.48, 1.32) & (0.43, 1.23) & & (0.42, 1.21) & \\
\(\gamma\)-Linolenic acid, & & & & & & \\
18:3 n-6 & 0.97 & 0.98 & 0.840 & 0.98 & 0.835 \\
 & & (0.80, 1.17) & (0.81, 1.19) & & (0.81, 1.19) & \\
Dihomolinoleic acid, & & & & & & \\
20:2 n-6 & 1.06 & 1.13 & 0.226 & 1.16 & 0.197 \\
 & & (0.87, 1.30) & (0.93, 1.38) & & (0.93, 1.44) & \\
Dihomo-\(\gamma\)-linolenic & & & & & & \\
acid, 20:3 n-6 & 1.40 & 1.34 & 0.037 & 1.34 & 0.035 \\
 & & (1.06, 1.85) & (1.02, 1.76) & & (1.02, 1.76) & \\
Arachidonic acid, & & & & & & \\
20:4 n-6 & 0.82 & 0.83 & 0.131 & 0.84 & 0.152 \\
 & & (0.65, 1.04) & (0.65, 1.06) & & (0.66, 1.07) & \\
Adrenic acid, 22:4 n-6 & & & & & & \\
 & 1.05 & 1.04 & 0.593 & 1.02 & 0.755 \\
 & & (0.91, 1.21) & (0.90, 1.21) & & (0.88, 1.19) & \\
Docosapentaenoic acid, 22:5n-6 & & & & & & \\
 & 0.88 & 0.95 & 0.630 & 0.96 & 0.716 \\
 & & (0.70, 1.09) & (0.76, 1.18) & & (0.77, 1.20) & \\
n-3 PUFA & & & & & & \\
\(\alpha\)-linolenic acid, & & & & & & \\
18:3n-3 & 0.94 & 0.736 & 1.05 & 0.759 & 1.06 \\
 & & (0.67, 1.32) & (0.75, 1.48) & & (0.76, 1.48) & \\
Stearidonic acid, & & & & & & \\
18:4n-3 & 0.92 & 0.89 & 0.184 & 0.91 & 0.274 \\
 & & (0.78, 1.09) & (0.75, 1.06) & & (0.77, 1.08) & \\
Eicosapentaenoic acid, 20:5n-3 & & & & & & \\
 & 1.11 & 1.07 & 0.586 & 1.06 & 0.633 \\
 & & (0.88, 1.39) & (0.85, 1.35) & & (0.84, 1.34) & \\
Docosapentaenoic acid, 22:5n-3 & & & & & & \\
 & 1.10 & 1.18 & 0.343 & 1.16 & 0.397 \\
 & & (0.79, 1.53) & (0.84, 1.66) & & (0.82, 1.64) & \\
Docosahexaenoic acid, 22:6n-3 & & & & & & \\
 & 0.78 & 0.77 & 0.064 & 0.78 & 0.081 \\
 & & (0.60, 1.03) & (0.58, 1.02) & & (0.59, 1.03) & \\
\textit{trans} fatty acid & & & & & & \\
\end{tabular}
\end{table}
trans-7-hexadecenoic acid, 16:1n-9t | 1.30 (0.88, 1.90) | 0.185 | 1.27 (0.85, 1.88) | 0.240 | 1.28 (0.86, 1.91) | 0.218

Palmitelaidic acid, 16:1n-7t | 1.03 (0.71, 1.50) | 0.865 | 0.96 (0.66, 1.41) | 0.843 | 0.98 (0.67, 1.43) | 0.907

Petroselinic acid, 18:1n-10-12t§ | 0.96 (0.62, 1.48) | 0.846 | 0.91 (0.58, 1.42) | 0.672 | 0.91 (0.58, 1.41) | 0.666

Elaidic acid, 18:1n-9t | 1.13 (0.80, 1.62) | 0.486 | 1.24 (0.86, 1.78) | 0.256 | 1.23 (0.86, 1.76) | 0.269

trans-Vaccenic acid, 18:1n-7t | 1.04 (0.73, 1.46) | 0.847 | 0.99 (0.70, 1.41) | 0.965 | 1.02 (0.72, 1.45) | 0.916

Linoelaidic acid, 18:2t‖ | 1.00 (0.88, 1.14) | 0.996 | 1.04 (0.91, 1.18) | 0.587 | 1.04 (0.91, 1.18) | 0.593

Conjugated linoleic acid, 18:2CLA# | 1.04 (0.85, 1.26) | 0.733 | 1.01 (0.83, 1.23) | 0.928 | 0.98 (0.80, 1.20) | 0.839

Values are hazard ratio (95% confidence interval) per standard deviation (n=1,681). All 35 individual NEFAs were included in a single model in the Cox proportional hazard regression to estimate the risk of CHD mortality with one standard deviation increment of each NEFA. CI, confidence interval; HR, hazard ratio; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SD, standard deviation; SFA, saturated fatty acid. *Model 1 adjusted for age, sex, race, field center, education, and all other 35 NEFAs; †Model 2 adjusts for model 1 covariates plus smoking status, serum albumin, alcohol consumption, cystatin C for estimate glomerular filtration rate, weight, height, and physical activity; ‡Model 3 adjusts for Model 2 covariates plus hypertension and diabetes; §18:1n10-12t, sum of 18:2n-10, n-11, and n-12 trans isomers; ‖18:2t, sum of all 18:2 trans isomers; #18:2CLA, conjugated linoleic acid.
Table S6. Multivariable adjusted hazard ratios relating 35 individual serum NEFAs with incident non-fatal myocardial infarction (MI) in the Cardiovascular Health Study cohort at baseline in 1996-1997.

| NEFAs, µmol/L per SD | Model 1* | Model 2† | Model 3‡ |
|----------------------|----------|----------|----------|
|                      | HR (95% CI) | P-value  | HR (95% CI) | P-value  | HR (95% CI) | P-value  |
| **SFA**              |          |          |          |          |          |          |
| Lauric acid, 12:0    | 1.06 (0.93, 1.20) | 0.408 | 1.05 (0.92, 1.20) | 0.442 | 1.05 (0.92, 1.20) | 0.446 |
| Myristic acid, 14:0  | 1.26 (0.80, 1.99) | 0.327 | 1.18 (0.75, 1.87) | 0.476 | 1.16 (0.73, 1.83) | 0.529 |
| Pentadecylic acid, 15:0 | 0.78 (0.54, 1.13) | 0.193 | 0.77 (0.53, 1.12) | 0.167 | 0.78 (0.54, 1.14) | 0.198 |
| Palmitic acid, 16:0  | 0.96 (0.62, 1.49) | 0.858 | 0.96 (0.61, 1.50) | 0.845 | 0.92 (0.59, 1.44) | 0.723 |
| Stearic acid, 18:0   | 1.11 (0.80, 1.54) | 0.544 | 1.15 (0.83, 1.61) | 0.402 | 1.16 (0.83, 1.61) | 0.390 |
| Arachidic acid, 20:0 | 0.93 (0.74, 1.19) | 0.580 | 0.96 (0.76, 1.23) | 0.762 | 0.97 (0.76, 1.24) | 0.798 |
| Behenic acid, 22:0   | 0.96 (0.81, 1.14) | 0.629 | 0.95 (0.79, 1.14) | 0.605 | 0.94 (0.79, 1.13) | 0.538 |
| Lignoceric acid, 24:0 | 1.01 (0.94, 1.09) | 0.708 | 1.02 (0.94, 1.10) | 0.702 | 1.01 (0.94, 1.09) | 0.733 |
| **MUFA**             |          |          |          |          |          |          |
| Myristoleic acid, 14:1n-5 | 0.90 (0.59, 1.35) | 0.599 | 0.90 (0.60, 1.35) | 0.611 | 0.91 (0.61, 1.37) | 0.656 |
| cis-7-hexadecenoic acid, 16:1n-9 | 1.10 (0.74, 1.64) | 0.633 | 1.11 (0.74, 1.66) | 0.608 | 1.14 (0.76, 1.69) | 0.531 |
| Palmitoleic acid, 16:1n-7 | 0.99 (0.59, 1.66) | 0.964 | 1.08 (0.63, 1.85) | 0.778 | 1.12 (0.66, 1.91) | 0.671 |
| Oleic acid, 18:1n-9   | 0.45 (0.21, 0.98) | 0.044 | 0.48 (0.22, 1.03) | 0.060 | 0.47 (0.22, 1.03) | 0.059 |
|                        | cis-Vaccenic acid, 18:1n-7 |  | Gondoic acid, 20:1n-9 |  | Erucic acid, 22:1n-9 |  | Nervonic acid, 24:1n-9 |  |
|------------------------|-----------------------------|---|-----------------------|---|----------------------|---|------------------------|---|
|                        |                             |   |                       |   |                      |   |                        |   |
|                        | 1.29                        | 0.395 | 1.17                  | 0.615 | 1.12                 | 0.715 |                      |   |
|                        | (0.71, 2.34)                |      | (0.64, 2.14)          |      | (0.61, 2.05)         |      | (0.71, 1.50)          |      |
|                        | 0.99                        | 0.972 | 1.00                  | 0.987 | 1.03                 | 0.885 |                      |   |
|                        | (0.67, 1.47)                |      | (0.69, 1.44)          |      | (0.71, 1.50)         |      | (0.71, 1.18)          |      |
|                        | 1.01                        | 0.859 | 1.02                  | 0.817 | 1.01                 | 0.894 |                      |   |
|                        | (0.87, 1.18)                |      | (0.88, 1.18)          |      | (0.87, 1.18)         |      | (0.87, 1.18)          |      |
|                        | 1.09                        | 0.063 | 1.09                  | 0.072 | 1.09                 | 0.088 |                      |   |
|                        | (1.00, 1.20)                |      | (0.99, 1.21)          |      | (0.99, 1.21)         |      | (0.99, 1.21)          |      |
| **n-6 PUFA**            |                             |   |                       |   |                      |   |                        |   |
|                        |                             |   |                       |   |                      |   |                        |   |
| Linoleic acid, 18:2n-6 | 1.66                        | 0.044 | 1.47                  | 0.140 | 1.46                 | 0.156 |                      |   |
|                        | (1.01, 2.72)                |      | (0.88, 2.46)          |      | (0.87, 2.45)         |      | (0.87, 2.45)          |      |
| γ-Linolenic acid, 18:3n-6 | 0.94                      | 0.504 | 0.96                  | 0.702 | 0.96                 | 0.690 |                      |   |
|                        | (0.78, 1.13)                |      | (0.80, 1.16)          |      | (0.80, 1.16)         |      | (0.80, 1.16)          |      |
| Dihomolinoleic acid, 20:2n-6 | 0.89                  | 0.412 | 0.93                  | 0.630 | 0.92                 | 0.612 |                      |   |
|                        | (0.67, 1.18)                |      | (0.70, 1.24)          |      | (0.68, 1.26)         |      | (0.68, 1.26)          |      |
| Dihomo-γ-Linolenic acid, 20:3n-6 | 1.06                  | 0.654 | 1.06                  | 0.660 | 1.06                 | 0.686 |                      |   |
|                        | (0.81, 1.39)                |      | (0.81, 1.39)          |      | (0.81, 1.38)         |      | (0.81, 1.38)          |      |
| Arachidonic acid, 20:4n-6 | 0.88                      | 0.298 | 0.86                  | 0.209 | 0.88                 | 0.313 |                      |   |
|                        | (0.70, 1.12)                |      | (0.67, 1.09)          |      | (0.69, 1.12)         |      | (0.69, 1.12)          |      |
| Adrenic acid, 22:4n-6  | 0.96                        | 0.604 | 0.92                  | 0.331 | 0.91                 | 0.276 |                      |   |
|                        | (0.83, 1.12)                |      | (0.77, 1.09)          |      | (0.76, 1.08)         |      | (0.76, 1.08)          |      |
| Docosapentaenoic acid, 22:5n-6 | 1.09                  | 0.415 | 1.13                  | 0.217 | 1.13                 | 0.219 |                      |   |
|                        | (0.89, 1.32)                |      | (0.93, 1.37)          |      | (0.93, 1.37)         |      | (0.93, 1.37)          |      |
| **n-3 PUFA**            |                             |   |                       |   |                      |   |                        |   |
|                        |                             |   |                       |   |                      |   |                        |   |
| α-Linolenic acid, 18:3n-3 | 0.83                      | 0.254 | 0.92                  | 0.644 | 0.93                 | 0.654 |                      |   |
|                        | (0.60, 1.15)                |      | (0.66, 1.30)          |      | (0.66, 1.30)         |      | (0.66, 1.30)          |      |
| Stearidonic acid, 18:4n-3 | 0.93                      | 0.397 | 0.91                  | 0.264 | 0.93                 | 0.384 |                      |   |
|                        | (0.79, 1.10)                |      | (0.77, 1.07)          |      | (0.79, 1.10)         |      | (0.79, 1.10)          |      |
| Eicosapentaenoic acid, 20:5n-3 | 1.11                  | 0.305 | 1.10                  | 0.365 | 1.08                 | 0.454 |                      |   |
|                        | (0.91, 1.37)                |      | (0.90, 1.35)          |      | (0.88, 1.33)         |      | (0.88, 1.33)          |      |
| Docosapentaenoic acid, 22:5n-3 | 1.15                  | 0.374 | 1.19                  | 0.293 | 1.19                 | 0.293 |                      |   |
|                        | (0.84, 1.58)                |      | (0.86, 1.64)          |      | (0.86, 1.65)         |      | (0.86, 1.65)          |      |
| Docosahexaenoic acid, 22:6n-3 | 0.81                  | 0.108 | 0.82                  | 0.134 | 0.82                 | 0.147 |                      |   |
|                        | (0.62, 1.05)                |      | (0.63, 1.06)          |      | (0.63, 1.07)         |      | (0.63, 1.07)          |      |

**trans fatty acid**
| NEFA | Hazard Ratio (95% CI) | Value | Hazard Ratio (95% CI) | Value | Hazard Ratio (95% CI) | Value |
|------|-----------------------|------|-----------------------|------|-----------------------|------|
| **trans-7-hexadecenoic acid, 16:1n-9t** | 0.96 (0.65, 1.40) | 0.94 (0.63, 1.40) | 0.749 (0.63, 1.41) | 0.94 (0.63, 1.40) | 0.764 (0.65, 1.40) |
| Palmitelaidic acid, 16:1n-7t | 1.29 (0.89, 1.88) | 1.27 (0.87, 1.84) | 0.222 (0.88, 1.87) | 1.28 (0.89, 1.88) | 0.203 (0.89, 1.88) |
| Petrosealinic acid, 18:1n-10-12t§ | 1.04 (0.67, 1.63) | 0.94 (0.60, 1.48) | 0.786 (0.61, 1.50) | 0.96 (0.63, 1.41) | 0.846 (0.61, 1.50) |
| **Elaidic acid, 18:1n-9t** | **1.40 (0.98, 2.02)** | **0.069 (1.06, 2.22)** | **0.025 (1.01, 2.12)** | **1.46 (0.98, 2.02)** | **0.045 (1.01, 2.12)** |
| trans-Vaccenic acid, 18:1n-7t | 0.74 (0.52, 1.06) | 0.75 (0.52, 1.06) | 0.106 (0.54, 1.10) | 0.77 (0.52, 1.06) | 0.150 (0.54, 1.10) |
| Linoelaidic acid, 18:2∥ | 0.96 (0.83, 1.10) | 1.00 (0.87, 1.16) | 0.955 (0.87, 1.16) | 1.01 (0.87, 1.16) | 0.946 (0.87, 1.16) |
| Conjugated linoleic acid, 18:2CLA# | 1.08 (0.89, 1.31) | 1.09 (0.90, 1.32) | 0.398 (0.88, 1.30) | 1.07 (0.89, 1.31) | 0.528 (0.88, 1.30) |

Values are hazard ratio (95% confidence interval) per standard deviation (n=1,681). All 35 individual NEFAs were included in a single model in the Cox proportional hazard regression to estimate the risk of incident non-fatal MI with one standard deviation increment of each NEFA.

CI, confidence interval; HR, hazard ratio; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SD, standard deviation; SFA, saturated fatty acid. *Model 1 adjusted for age, sex, race, field center, education, and all other 35 NEFAs; †Model 2 adjusts for model 1 covariates plus smoking status, serum albumin, alcohol consumption, cystatin C for estimate glomerular filtration rate, weight, height, and physical activity; ‡Model 3 adjusts for Model 2 covariates plus hypertension and diabetes; §18:1n10-12t, sum of 18:2n-10, n-11, and n-12 trans isomers; ∥18:2t, sum of all 18:2 trans isomers; #18:2CLA, conjugated linoleic acid.
Figure S1. The correlations between all 35 non-esterified fatty acids.