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Plasma Phospholipid *Trans*-Fatty Acids Levels, Cardiovascular Diseases, and Total Mortality: The Cardiovascular Health Study

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Background—While self-reported *trans*-fatty acid (TFA) consumption is linked to coronary heart disease (CHD), relationships between objective biomarkers of TFA subtypes (*t*-16:1n9, total *t*-18:1, and *cis/trans*-(*c/t*), *t/c*- and *t/t*-18:2) and cardiovascular disease (CVD) or total mortality are not well established.

Methods and Results—We evaluated 2742 adults in the Cardiovascular Health Study, aged 74±5 years and free of prevalent CVD, with plasma phospholipid TFA measures in 1992. Incident fatal and nonfatal CHD events, CVD and non-CVD mortality, and total mortality were centrally adjudicated through 2010. Risks were assessed using Cox proportional hazards. During 31 494 person-years, 1735 total deaths and 639 total CHD events occurred. In the multivariate model including mutual adjustment for the 5 TFA subtypes, circulating *t/t*-18:2 was associated with higher total mortality (extreme quintile hazard ratio (HR)=1.23, 95% CI=1.04 to 1.44, *P*-trend=0.01), CVD mortality (HR=1.40, 95% CI=1.05 to 1.86, *P*-trend=0.02), and total CHD (HR=1.39, 95% CI=1.06 to 1.83, *P*-trend=0.01). *t/c*-18:2 was positively related to total mortality (HR=1.19, *P*-trend=0.05), total CHD (HR=1.67, *P*-trend=0.002), and nonfatal CHD (HR=2.06, *P*-trend=0.002) after mutual adjustment; these associations were insignificant without mutual adjustment. Neither *t*-16:1n9 nor *t*-18:1 was significantly associated with total mortality or CVD, nor was *c/t*-18:2 if we excluded early cases.

Conclusions—Among circulating TFAs, *t/t*-18:2 was most adversely associated with total mortality, mainly due to the increased risk of CVD. *t/c*-18:2 was also positively associated with total mortality and CHD, but only after adjustment for other TFAs. These results highlight the need for further investigation of dietary sources, nondietary determinants, and health effects of specific TFA subtypes, especially *t*-18:2 isomers. (*J Am Heart Assoc.* 2014;3:e000914 doi: 10.1161/JAHA.114.000914)

Key Words: cardiovascular diseases • coronary disease • nutrition • total mortality • *trans*-fatty acids

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The US Food and Drug Administration recently announced a preliminary determination that partially hydrogenated vegetable oil (PHVO), the primary source of industrial *trans*-fatty acids (TFA) in processed foods, will no longer be “generally recognized as safe” for use in food.¹ This was based on evidence that consumption of TFA (unsaturated fatty acids with 1 or more carbon double-bonds in the *trans* configuration) is associated with risk of coronary heart disease (CHD), adverse lipid effects,^{2–5} endothelial dysfunction,^{6,7} and pro-inflammatory responses.^{6,8–10} The US Food and Drug Administration's action and most research to date have focused on total TFA, predominantly representing *t*-18:1 (an 18-carbon fatty acid with a single *trans* double-bond).^{11–16} However, different TFA subtypes (of varying chain length and unsaturation) and different TFA isomers (of similar chain length and unsaturation, but different location of double bonds) may have both divergent dietary sources¹⁷ and health effects.^{18–22} For example, prior case-control studies suggested that *trans*-isomers of linoleic acid (*t*-18:2), but not of oleic acid (*t*-18:1) or palmitoleic acid (*t*-16:1), were associated with CHD death and sudden cardiac arrest.^{18,19} How exposure to these different TFA subtypes

relates to other key outcomes, such as total mortality or non-cardiovascular disease (CVD) mortality, is not established. In addition, although circulating *t*-18:2 appear especially relevant for CVD risk,^{18,19,22} prior studies evaluated only the sum of these fatty acids, and it is unknown how different individual *t*-18:2 isomers, including *c/t*-, *t/c*-, and *t/t*-18:2n6,9 (*t/t*-18:2), relate to CHD, CVD death, and total mortality.

To address these key gaps in knowledge, we investigated prospective associations of plasma phospholipid TFA levels, objective biomarkers of exposure, with total mortality, CVD and non-CVD mortality, and CHD incidence in the Cardiovascular Health Study (CHS), a community-based prospective cohort of older US adults. Based on evidence from mechanistic studies and effects on risk factors,^{18,19,22} we hypothesized that *t*-18:2, especially *t/t*-18:2, would be associated with greater mortality and CVD risk, whereas *t*-18:1 and *t*-16:1n9 would not be. In addition, we hypothesized that circulating TFA subtypes would not be significantly associated with non-CVD mortality. Given potentially diverse dietary sources of different TFA subtypes and isomers,^{16,23} establishing their health effects is crucial for both scientific understanding of how fatty acids influence health as well as for informing policy priorities.

Methods

Design and Population

CHS is a National Heart, Lung, and Blood Institute-sponsored, community-based, multicenter prospective cohort of older US adults.²⁴ Briefly, 5201 ambulatory, noninstitutionalized adults aged 65 and over were randomly selected and enrolled in 1989–1990 from Medicare eligibility lists in 4 US communities (Forsyth County, NC; Sacramento County, CA; Washington County, MD; Allegheny County, PA); an additional 687 black participants were similarly recruited and enrolled in 1992–1993. Among all eligible adults contacted, 57% agreed to enroll. Each center's institutional review committee approved the study, and all participants provided informed written consent.

Among cohort participants alive at the 1992–1993 study visit, we measured fatty acids in 3941 (71% of 5553) participants from blood samples drawn in 1992–1993, considered the baseline year for this analysis. For the present analysis, we excluded 1199 participants with prevalent CVD (coronary heart disease, stroke, transient ischemic attack, congestive heart failure, or atrial fibrillation) at this visit, resulting in 2742 participants evaluated in this analysis.

Fatty Acid Measurements

Plasma phospholipid fatty acid compositions were measured at the Fred Hutchinson Cancer Research Center using

previously described methods (Data S2: Fatty acid measurements).²⁵ Total lipids were extracted from plasma using the method of Folch,²⁶ and the phospholipid fraction was isolated by 1-dimensional thin layer chromatography. Fatty acid methyl esters were prepared by direct transesterification and separated using gas chromatography to quantify 45 distinct fatty acid peaks. Measured TFAs included 16-carbon monounsaturated (*t*-16:1n7, *t*-16:1n9), 18-carbon monounsaturated (*t*-18:1n6, 18:1n7, 18:1n8, 18:1n9, 18:1n10-12), and 18-carbon polyunsaturated (*t/t*-, *c/t*-, *t/c*-18:2) isomers. Given our focus on the industrial TFAs, we decided a priori to evaluate *t*-16:1n7 in a separate investigation, due to its non-PHVO dietary sources¹⁷ and inverse associations with metabolic risk.^{27,28} For this analysis, we evaluated *t*-16:1n9, the 3 *t*-18:2 isomers, and the sum of the *t*-18:1 isomers (*t*-18:1n6, 18:1n7, 18:1n8, 18:1n9, 18:1n10-12), given their very high intercorrelations ($r > 0.83$). These selected 5 TFA subtypes reflect a comprehensive list of the known TFAs coming from PHVO. Laboratory coefficients of variation were 5% for *t*-16:1n9, 2% for total *t*-18:1, and 8% for total *t*-18:2.

While all TFA measurements were performed using stored blood from 1992 to 1993, the laboratory assays were tested at 2 time points: in 1999–2002 for a prior case-control study of CHD, and in 2007–2012 for all available samples in the cohort. We developed a calibration equation to evaluate and account for potential laboratory drift using duplicate measurements across these 2 time periods in a subset of participants. Laboratory drift was identified for *t*-16:1n9 and *t/t*-18:2, and calibration equations were used to correct for potential drift (Data S1: Method of assessment and correction for laboratory drifts). We also performed sensitivity analyses excluding the 285 participants with earlier measures.

We assessed long-term reproducibility by comparing within-person TFA levels in 100 subjects across blood samples drawn in 1992–1993, 1998–1999, and 2005–2006. The 6-year and 13-year correlations with baseline levels were 0.53 and 0.46 for *t*-16:1n9, 0.65 and 0.60 for *t*-18:1, 0.54 and 0.48 for *c/t*-18:2, 0.35 and 0.22 for *t/c*-18:2, and 0.36 and 0.43 for *t/t*-18:2. The within-person correlations of 0.40 to 0.60 compare favorably to similar correlations over time for other common CVD risk factors such as blood pressure and cholesterol levels.^{29,30} Conversely, the lower within-person reliability for *t/c*-18:2 would cause greater exposure misclassification over time and attenuation of true associations toward the null.

Other Risk Factors

At baseline in 1992–1993, sociodemographic characteristics, medical history, and lifestyle risk factors were collected during a 90-minute home interview;²⁴ and body-mass index, waist circumference, and blood pressure were measured by

Table 1. Baseline Concentrations of Plasma Phospholipid *Trans*-Fatty Acid and Their Correlations in the Cardiovascular Health Study (n=2742)

	<i>Trans</i> -16:1n9	Total <i>Trans</i> -18:1*	<i>Cis/Trans</i> -18:2	<i>Trans/Cis</i> -18:2	<i>Trans/Trans</i> -18:2	Total <i>Trans</i> Fat†
Mean concentration (SD), % of fatty acids	0.07 (0.02)	1.97 (0.71)	0.08 (0.02)	0.13 (0.05)	0.05 (0.02)	2.48 (0.78)
5th, 95th percentiles	0.04, 0.11	0.96, 3.24	0.04, 0.12	0.07, 0.23	0.03, 0.07	1.19, 3.68
Pearson correlation						
<i>Trans</i> -16:1n9 (cv=5%)	1.00					
Total <i>trans</i> -18:1* (cv=2%)	0.75	1.00				
<i>Cis/trans</i> -18:2 (cv=8%)	0.47	0.62	1.00			
<i>Trans/cis</i> -18:2 (cv=8%)	0.33	0.50	0.78	1.00		
<i>Trans/trans</i> -18:2 (cv=8%)	0.08	0.09	-0.04	0.02	1.00	
Total <i>trans</i> fat† (cv=2%)	0.76	0.99	0.67	0.56	0.09	1.00

Cv indicates coefficient of variation from laboratory; SD, standard deviation.

*Total *trans*-18:1 consisted of subtypes including *trans*-18:1n6, n7, n8, n9, n10-12 with intercorrelation between each subtype ranging from 0.83 to 0.93.

†Total *trans*-fatty acids were sum of *trans*-16:1n7, *trans*-16:1n9, total *trans*-18:1, *cis/trans*-18:2, *trans/cis*-18:2, *trans/trans*-18:2.

standardized methods during a clinical examination. Fasting total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, and C-reactive protein (CRP)³¹ were measured centrally; and low-density lipoprotein (LDL) cholesterol was calculated according to the Friedewald equation.³² Dietary habits were assessed 3 years earlier, in 1989–1990, using a picture-sort food frequency questionnaire validated against 6 detailed 24-hour diet recall interviews spaced ≈1 month apart.^{33,34}

Ascertainment of Events

Participants were followed by means of annual study-clinic examinations with interim phone contacts for 10 years, and telephone contacts every 6 months thereafter. Follow-up for vital status was 100% complete; <1% of all person-time was missing and censored early. All potential CVD events and deaths were reviewed and classified by centralized adjudication committees of physicians using information from participant or proxy interviews, medical records, physician questionnaires, death certificates, medical examiner forms, Centers for Medicare & Medicaid Services hospitalizations, and available diagnostic tests and consultations. Algorithms and methods for mortality follow-up and confirmation and classification of CHD in CHS have been described.^{35,36} CVD mortality was defined as death due to atherosclerotic CHD, cerebrovascular disease, other atherosclerotic disease, and other CVD. Total incident CHD was also adjudicated, including CHD death and nonfatal myocardial infarction. Given the large number of clinical end points examined and the complexities of evaluating total and subtypes of stroke, we did not investigate stroke in this study. However, due to the substantial biological and clinical relevance of stroke as well

as the heterogeneity in stroke phenotypes, we are going to separately evaluate stroke as an end point in another study.

Statistical Analysis

Fatty acids levels were evaluated in quintiles as categorical variables. Tests for trend were evaluated by assigning each participant the median value of each quintile category and evaluating this as a continuous variable. Cox proportional-hazards were used to estimate hazard ratios of events, with time-at-risk until the first event of interest, censoring at other causes of death in analyses of cause-specific mortality or at the latest date of adjudicated follow-up. The proportional hazard assumption was not rejected on the basis of Schoenfeld residuals for each TFA biomarker. To minimize potential confounding, we adjusted for age, sex, race, education, enrollment site, diabetes, hypertension, smoking, alcohol consumption, physical activity, body-mass index, waist circumference, phospholipid omega-6 and omega-3 fatty acids, and estimated dietary saturated and monounsaturated fat consumption. Because alcohol consumption could be nonlinearly related to CHD,^{37,38} we also modeled alcohol consumption with quadratic terms or as categorical variable (<1, 1 to 14, 14+ servings/week) in the multivariate model. In addition, we further adjusted for levels of LDL cholesterol, HDL cholesterol, and CRP as potential mediators in the multivariate model, because circulating TFAs could influence the levels of LDL cholesterol, HDL cholesterol, and CRP, which could further affect the risk of total mortality and CVD. The extent of attenuation in the effect estimates after the adjustment can help reflect the effects of TFAs attributable to the mediators in the model.³⁹ Missing covariate information (most factors <2%; dietary factors = 4% to 10%) was imputed by best-subset-

Table 2. Baseline Characteristics of the 2742 Participants, by Quintile of Each Plasma Phospholipid *Trans*–Fatty Acid Concentration

	<i>Trans</i> -16:1n9		Total <i>Trans</i> -18:1 [†]		<i>Cis</i> / <i>Trans</i> -18:2		<i>Trans</i> / <i>Cis</i> -18:2		<i>Trans</i> / <i>Trans</i> -18:2	
	I	V	I	V	I	V	I	V	I	V
Range, % total fatty acids	0.02–0.05	0.09–0.20	0.23–1.37	2.55–5.70	0.01–0.06	0.10–0.26	0.02–0.09	0.17 to 0.62	0.011 to 0.036	0.053 to 0.085
N adults in quintile	550	548	549	548	550	528	562	548	594	536
Age, y	73.0±4.6	74.7±5.4*	73.5±4.9	74.7±5.4*	74.2±5.3	74.2±5.4	73.8±5.1	73.9±4.9	73.0±4.6	74.3±5.4*
Gender, %male	38	33	36	37	47	34*	41	36	31	39*
Race, %white	87	92*	81	93*	73	97*	78	93*	94	69*
Education, % >high school	51	41*	53	36*	46	42	48	43	46	47
Diabetes mellitus [‡] , %	11	10	16	10*	20	8*	19	11*	12	16
Hypertension [§] , %	41	36	43	38	41	35	43	35	38	45
Current smoker, %	10	11	10	12	9	10	10	12	10	10
Former smoker, %	45	38	48	37*	47	42	43	44	43	42
Alcohol, drinks/week	4.4±7.2	0.9±2.6*	5.1±12.1	0.9±3.7*	2.8±5.9	1.5±4.4*	2.94±10.8	1.64±4.4*	3.3±11.0	1.6±4.2*
Physical activity, kcal/week	1318±1803	901±1150*	1126±1619	947±1194	1167±1537	1127±1491	1141±1602	1020±1291	999±1243	1032±1420
Body–mass index, kg/m ²	27.1±4.4	25.9±4.1*	27.1±4.7	25.6±4.2*	26.8±4.6	26.6±4.6	26.4±4.5	26.8±4.7	27.6±4.6	26.0±4.7*
Waist circumference, cm	97.8±13.4	95.2±11.8*	97.9±13.8	94.7±11.9*	97.2±13.4	96.8±12.9	96.2±13.2	97.4±13.0	98.8±13.1	93.9±13.5*
Systolic blood pressure, mm Hg	135.2±20.1	137.9±22.1	135.7±19.1	138.1±22.4	135.2±20.1	137.7±21.2	134.9±20.2	137.7±21.5	135.1±20.2	137.8±21.8
Diastolic blood pressure, mm Hg	72.2±11.0	71.2±11.3	72.0±11.6	70.8±11.0	71.9±11.7	71.1±11.4	71.4±11.2	71.7±11.3	71.2±12.1	72.4±11.5
LDL cholesterol, mg/dL	125.8±34.0	130.0±32.9	124.4±36.3	128.6±32.3*	127.6±34.7	125.9±32.8	125.5±33.1	129.2±33.8	127.9±33.5	124.1±34.7
HDL cholesterol, md/dL	56.8±15.5	53.2±13.2*	58.0±16.1	53.1±14.0*	55.0±14.4	54.0±14.3	55.1±14.6	53.2±13.9	53.4±14.2	57.7±14.9*
Triglyceride, mg/dL	144.6±95.8	135.3±72.8	143.5±93.8	138.8±80.4	124.8±69.7	156.7±88.2*	127.0±69.6	156.8±91.1*	180.1±99.5	109.2±71.4*
C-reactive protein, mg/L	6.0±10.1	3.4±5.2*	5.7±10.1	4.1±9.5*	5.6±11.0	4.6±9.7	5.5±11.6	4.7±8.5	5.4±9.3	4.9±11.5
Dietary factors										
SFA, %energy	10.5±2.2	10.0±2.1*	10.5±2.3	10.2±2.1	10.4±2.2	10.2±2.1	10.2±2.3	10.3±2.1	10.3±2.3	10.3±2.0
MUFA, %energy	11.6±2.3	11.4±2.4	11.5±2.4	11.6±2.4	11.5±2.4	11.5±2.4	11.4±2.4	11.7±2.4	11.6±2.4	11.7±2.2
Carbohydrates, %energy	51.3±7.5	53.4±7.9*	51.5±7.9	52.8±7.6*	51.8±7.5	53.2±7.9*	52.3±7.7	52.6±7.9	52.1±8.0	52.0±7.1
Fiber, g/day	28.9±12.5	30.3±12.2	29.1±12.3	29.1±12.3	29.2±12.0	29.6±12.4	30.0±12.0	29.0±12.1	29.2±12.8	29.7±11.0
<i>Trans</i> 16:1, %energy	0.08±0.03	0.07±0.03*	0.08±0.03	0.07±0.03	0.08±0.03	0.07±0.03	0.07±0.03	0.08±0.03*	0.08±0.03	0.08±0.03

Continued

Table 2. Continued

	Trans-16:1n9		Total Trans-18:1 [†]		Cis-/Trans-18:2		Trans-/Cis-18:2		Trans-/Trans-18:2	
	I	V	I	V	I	V	I	V	I	V
Trans18:1, %energy	1.91±0.6	2.09±0.7*	1.87±0.6	2.16±0.7*	1.93±0.7	2.13±0.7*	1.88±0.7	2.15±0.7*	1.96±0.6	2.03±0.7
Trans18:2, %energy	0.15±0.05	0.16±0.06*	0.10±0.05	0.20±0.06*	0.15±0.06	0.16±0.06*	0.14±0.06	0.17±0.06*	0.15±0.06	0.16±0.06
Plasma phospholipid biomarker										
Linoleic acid	18.8±2.6	20.3±2.4*	18.5±2.5	20.7±2.3*	18.9±2.5	20.2±2.4*	19.1±2.6	19.9±2.4*	19.5±2.6	19.5±2.4
EPA+DHA	3.8±1.2	3.5±1.3*	4.0±1.4	3.2±1.0*	4.1±1.4	3.1±0.9*	4.1±1.4	3.2±1.0*	3.5±1.1	3.8±1.3*

Baseline characteristics among adults in the lowest and highest quintile categories for each trans-fatty acid level are presented (values for other quintiles are presented in Table S3). Values for continuous variable represent mean±standard deviation; values for categorical variables represent percentage. EPA+DHA indicates eicosapentaenoic acids plus docosahexaenoic acids; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MUFA, monounsaturated fatty acids; SFA, saturated fatty acids.

*P<0.01 for trend across quintile categories are highlighted by asterisk.

[†]Total trans-18:1 consisted of subtypes including trans-18:1n6, n7, n8, n9, n10-12 that were highly inter-related (pairwise correlations=0.83 to 0.93). Total trans-fatty acids predominantly consisted of trans-18:1 (r=0.99), findings for total fats were similar to findings for total trans-18:1 (data not shown).

[‡]Diabetes mellitus was defined as a fasting glucose value ≥126 mg/dL or use of insulin or oral hypoglycemic agent.

[§]Hypertension was defined as systolic and diastolic blood pressures ≥140/90 mm Hg or use of antihypertensive treatment.

^{||}Diet factors were assessed 3 years before baseline in year 1989–1990.

regression using multiple demographic/risk variables. Potential nonlinear associations were evaluated using restricted cubic splines.⁴⁰

Because different TFA subtypes shared some common dietary sources and were partly intercorrelated,¹⁷ associations for 1 subtype could be confounded by the influence of other subtypes.¹⁹ We therefore performed analyses with and without mutual adjustment for the various TFA subtypes. We also performed sensitivity analyses evaluating total t-18:2 (summing c/t-, t/c-, and t/t-18:2); censoring the cohort at the midpoint of follow-up (8 years) to minimize misclassification due to exposure variation over time; and excluding events within the first 2 years of follow-up to minimize reverse causation from pre-existing subclinical disease. We also performed analyses corrected for regression dilution due to within-individual variation in TFA levels over time to obtain estimates of association due to usual levels of TFA exposure.⁴¹ We explored potential effect modification by age, sex, or race using multiplicative interaction terms and likelihood ratio testing, with Bonferroni correction for these exploratory multiple comparisons. Analyses were performed using Stata 10.1, with 2-tailed α=0.05 except for exploratory tests for effect modification (2-tailed α=0.00056).

Results

At baseline, mean±SD age was 74±5.1 years; 63.6% of participants were women, and 12.3% were African American. Intercorrelations between TFA isomers varied and were lowest for t/t-18:2 (Table 1). Due to higher concentrations, total t-18:1 correlated most strongly with total TFA.

In unadjusted cross-sectional analyses at baseline, higher t-16:1n9 and t-18:1 levels were associated with older age and lower education (Table 2). Most TFAs were associated with white race, except for t/t-18:2, which was associated with black race. All TFAs correlated with lower alcohol consumption; associations with other lifestyle factors such as smoking status and physical activity were more modest. t-16:1n9, t-18:1 and t/t-18:2 were inversely associated with body-mass index and waist circumference; while t-16:1n9 and t-18:1 were inversely and t/t-18:2 was positively associated with HDL cholesterol. In addition, c/t- and t/c-18:2 were positively and t/t-18:2 was inversely associated with triglycerides. Circulating TFA levels were generally unassociated with LDL-cholesterol levels, blood pressure, or CRP.

During 31 494 person-years of follow-up, 1735 deaths occurred. In multivariable-adjusted analysis, t/t-18:2 was associated with higher total mortality (Table 3), with 21% higher risk among participants in the top quintiles, compared with the bottom quintile (hazard ratio [HR]=1.21, 95% CI=1.04 to 1.32; P-trend=0.01). Other TFA subtypes were

Table 3. Prospective Associations of Plasma Phospholipid *Trans*-Fatty Acids With Risk of Total Mortality (n=2742)

	Quintiles of Plasma Phospholipids <i>Trans</i> -Fatty Acid Levels					P Trend*
	I	II	III	IV	V	
<i>Trans</i> -16:1n9						
Cases (person-years)	328 (6464)	328 (6478)	336 (6345)	306 (6089)	346 (6118)	
Hazard ratio (95% CI) [†]	1.00 (ref)	1.00 (0.86, 1.17)	0.99 (0.85, 1.15)	0.89 (0.76, 1.04)	1.02 (0.87, 1.19)	0.88
Hazard ratio (95% CI) [‡]	1.00 (ref)	1.05 (0.90, 1.23)	1.06 (0.89, 1.25)	0.93 (0.77, 1.13)	1.05 (0.85, 1.30)	0.92
Total <i>trans</i> -18:1 [§]						
Cases (person-years)	344 (6162)	311 (6411)	314 (6476)	321 (6348)	354 (6097)	
Hazard ratio (95% CI) [†]	1.00 (ref)	0.83 (0.72, 0.97)	0.81 (0.69, 0.94)	0.93 (0.80, 1.09)	0.90 (0.77, 1.06)	0.75
Hazard ratio (95% CI) [‡]	1.00 (ref)	0.83 (0.70, 0.99)	0.80 (0.66, 0.96)	0.91 (0.73, 1.13)	0.82 (0.64, 1.05)	0.33
<i>Cis/trans</i> -18:2						
Cases (person-years)	354 (5953)	306 (6375)	307 (6752)	345 (6511)	332 (5903)	
Hazard ratio (95% CI) [†]	1.00 (ref)	0.83 (0.71, 0.97)	0.83 (0.72, 0.97)	0.91 (0.78, 1.06)	0.98 (0.84, 1.15)	0.73
Hazard ratio (95% CI) [‡]	1.00 (ref)	0.87 (0.74, 1.02)	0.86 (0.71, 1.03)	0.90 (0.72, 1.10)	0.92 (0.72, 1.17)	0.65
<i>Trans/cis</i> -18:2						
Cases (person-years)	345 (6354)	317 (6304)	310 (6475)	320 (6282)	352 (6078)	
Hazard ratio (95% CI) [†]	1.00 (ref)	0.89 (0.77, 1.04)	0.92 (0.79, 1.07)	1.01 (0.86, 1.18)	1.08 (0.92, 1.26)	0.07
Hazard ratio (95% CI) [‡]	1.00 (ref)	0.97 (0.82, 1.14)	1.04 (0.87, 1.24)	1.13 (0.93, 1.37)	1.19 (0.96, 1.49)	0.05
<i>Trans/trans</i> -18:2						
Cases (person-years)	328 (7137)	302 (5898)	341 (6475)	330 (6113)	343 (5870)	
Hazard ratio (95% CI) [†]	1.00 (ref)	1.04 (0.89, 1.21)	1.07 (0.92, 1.24)	1.09 (0.94, 1.27)	1.21 (1.04, 1.42)	0.01
Hazard ratio (95% CI) [‡]	1.00 (ref)	1.05 (0.90, 1.23)	1.08 (0.92, 1.25)	1.09 (0.93, 1.27)	1.23 (1.04, 1.44)	0.02

BMI indicates body mass index; EPA+DHA, eicosapentaenoic acids plus docosahexaenoic acids.

*P-trend was evaluated by assigning each participant the median value of each quintile category and evaluating this as a continuous variable.

[†]Hazard ratios without mutual adjustment (95% CI) were adjusted for age (years), sex (male/female), race (white/nonwhite), education (<high school, high school, some college, college graduate), enrollment site, diabetes (yes/no), hypertension (yes/no), smoking (never/former/current), alcohol consumption (servings/week), physical activity (kcal/week), BMI (kg/m²), waist circumference (cm), dietary saturated fat (%energy), dietary monounsaturated fat (%energy), dietary fiber (g/day), plasma phospholipid EPA+DHA (%fatty acids), plasma phospholipid linoleic acid (%fatty acids).

[‡]Hazard ratios with mutual adjustment (95% CI) were adjusted for age (years), sex (male/female), race (white/nonwhite), education (<high school, high school, some college, college graduate), enrollment site, diabetes (yes/no), hypertension (yes/no), smoking (never/former/current), alcohol consumption (servings/week), physical activity (kcal/week), BMI (kg/m²), waist circumference (cm), dietary saturated fat (%energy), dietary monounsaturated fat (%energy), dietary fiber (g/day), plasma phospholipid EPA+DHA (%fatty acids), plasma phospholipid linoleic acid (%fatty acids), and other types of plasma phospholipid *trans*-fatty acids (% of fatty acids).

[§]Total *trans*-18:1 consisted of subtypes including *trans*-18:1n6, n7, n8, n9, n10-12 that were highly inter-related (pairwise correlations=0.83 to 0.93).

not significantly associated with total mortality. Modeling alcohol consumption with quadratic terms or as categorical variables, or further adjusting for CRP, LDL cholesterol, and HDL cholesterol did not appreciably alter these results (data not shown). In restricted cubic splines analyses, none of the circulating TFA subtypes were significantly nonlinearly related to total mortality (P -nonlinearity<0.05 for each).

Due to intercorrelations of the TFA subtypes (Table 1), we performed analyses with and without mutual adjustment to evaluate independent associations (Figure 1). After mutual adjustment, *t/t*-18:2 remained positively associated with total mortality (extreme quintile HR=1.23, P -trend=0.01, Table 3). In addition, *t/c*-18:2 was associated with a trend toward higher total mortality (HR=1.19, 95% CI=0.96 to 1.49, P -trend=0.05). Other circulating TFAs were not significantly

associated with total mortality either with or without mutual adjustment.

When we evaluated CVD and non-CVD mortality and incident CHD after mutual adjustment, *t/t*-18:2 was associated with 40% higher risk of CVD mortality (HR=1.40, 95% CI=1.05 to 1.86, P -trend=0.02, Table 4 and Figure 1), but not with non-CVD mortality (P -trend=0.14); and with 39% higher incidence of total CHD (HR=1.39, 95% CI=1.06 to 1.83, P -trend=0.01, Table 5 and Figure 1), with generally similar although not significant associations for fatal and nonfatal CHD (P -trend>0.08 for each). No other TFAs were significantly related to CVD or non-CVD mortality. *t/c*-18:2 was positively associated with total CHD (HR=1.67, 95% CI=1.17 to 2.40, P -trend=0.002) and nonfatal CHD (HR=2.06, 95% CI=1.29 to 3.28, P -trend=0.002), but not fatal CHD (P -trend=0.07). In

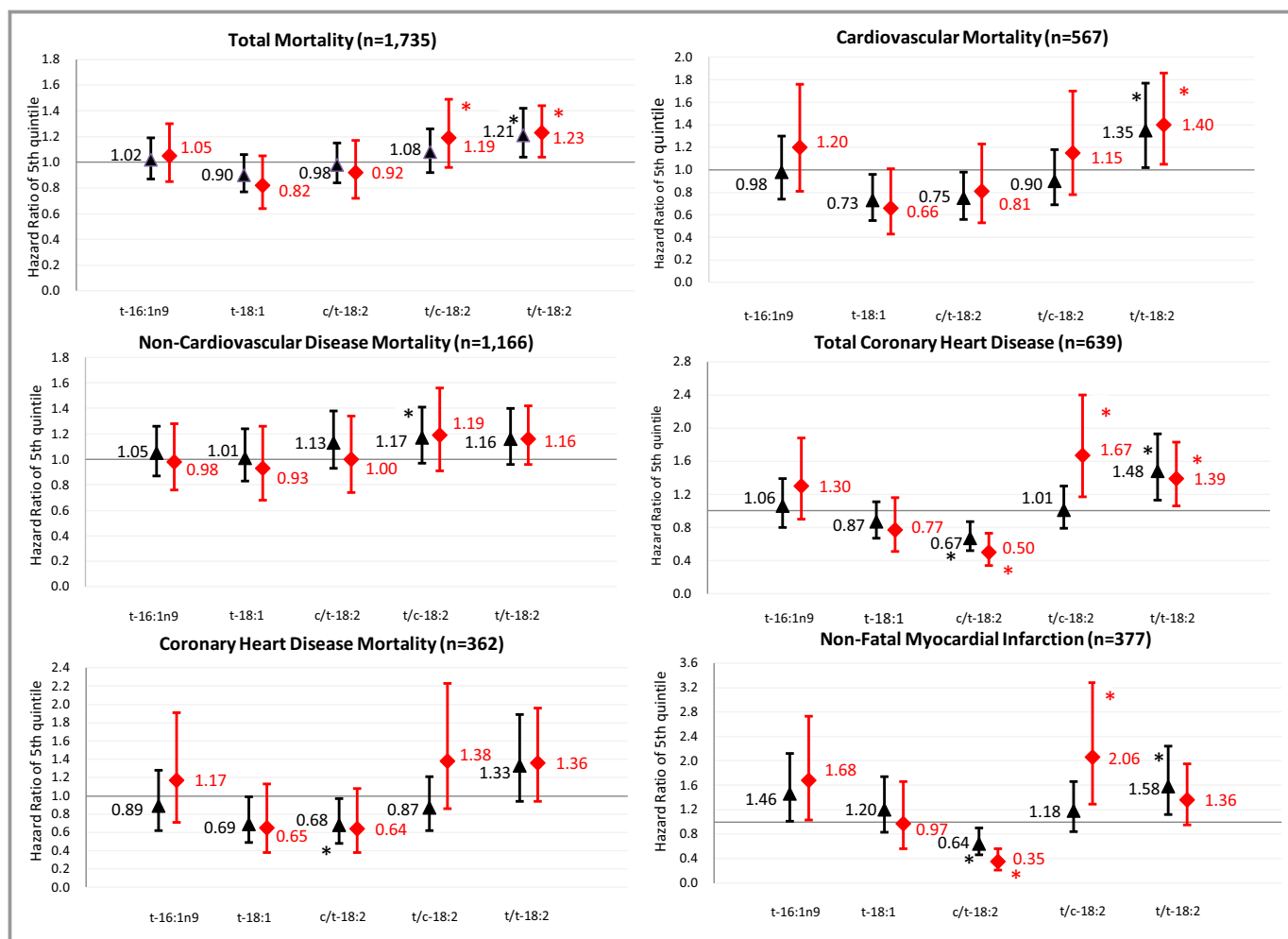


Figure 1. Prospective associations of plasma phospholipid TFA with total mortality, CVD, and CHD before and after mutual adjustment. Black triangles indicate multivariate-adjusted hazard ratios comparing adults in the top quintile category to adults in the bottom quintile category, before mutual adjustment for the 5 TFAs; red diamonds, after mutual adjustment for the 5 TFAs. Error bars represent 95% CI. All risk estimates were adjusted for age, sex, race, education, enrollment site, diabetes, hypertension, smoking, alcohol consumption, physical activity, BMI, waist circumference, dietary saturated fat, dietary monounsaturated fat, dietary fiber, plasma phospholipid EPA+DHA, plasma phospholipid linoleic acid. **P*-trend<0.05 across quintiles. BMI indicates body-mass index; CHD, coronary heart disease; CVD, cardiovascular disease; EPA+DHA, eicosapentaenoic acids plus docosahexaenoic acids; TFA, *trans*-fatty acid.

contrast, *c/t*-18:2 was inversely associated with total CHD (HR=0.50, 95% CI=0.34 to 0.73, *P*-trend=0.001) and nonfatal CHD (HR=0.35, 95% CI=0.21 to 0.56, *P*-trend<0.001), but not fatal CHD (*P*-trend=0.10). Neither *t*-16:1n9 nor *t*-18:1 was significantly associated with fatal or nonfatal CHD (*P*-trend>0.05 each).

Several of these associations were similar with (Tables 4, 5 and Figure 1) or without mutual adjustment (Tables 6 and 7). The main exceptions were for *c/t*- and *t/c*-18:2 (intercorrelation *r*=0.78), for which associations were generally strengthened with mutual adjustment. There was little evidence that any of the observed relationship were modified by age, sex, or race (*P*-interaction>0.001 each).

In sensitivity analyses, total *t*-18:2 (the sum of *c/t*, *t/c*, and *t/t* isomers) was positively associated with total

mortality (HR=1.24, 95% CI=1.03 to 1.49, *P*-trend=0.009) and non-CVD mortality (HR=1.24, 95% CI=1.03 to 1.62, *P*-trend=0.007), but not CVD mortality or CHD incidence (*P*-trend>0.31 each). Excluding events within the first 2 years of follow-up to minimize reverse causation had little effect on results, except for attenuation of the inverse associations between *c/t*-18:2 and total (HR=1.02, 95% CI=0.87 to 1.21, *P*-trend=0.36), fatal (HR=0.75, 95% CI=0.52 to 1.09, *P*-trend=0.14), and nonfatal CHD (HR=0.83, 95% CI=0.62 to 1.10, *P*-trend=0.33). Censoring follow-up at 8 years to minimize exposure misclassification with increasing duration of follow-up did not appreciably change the results (data not shown). We also corrected for regression dilution in TFA measurements; as would be expected, the magnitude of each of the associations was strengthened,

Table 4. Prospective Association of Plasma Phospholipid *Trans*-Fatty Acid With Cardiovascular and Noncardiovascular Mortality With Mutual Adjustment (n=2742)

	Quintiles of Plasma Phospholipid <i>Trans</i> -Fatty Acid Levels					P Value For Trend*
	I	II	III	IV	V	
Cardiovascular mortality (n=567)						
<i>Trans</i> 16:1n9	1.00 (ref)	1.37 (1.04, 1.80)	1.21 (0.89, 1.65)	1.16 (0.82, 1.63)	1.20 (0.81, 1.76)	0.65
Total <i>trans</i> 18: 1 [†]	1.00 (ref)	0.72 (0.54, 0.96)	0.64 (0.46, 0.89)	0.71 (0.48, 1.04)	0.66 (0.43, 1.01)	0.14
<i>Cis</i> <i>trans</i> 18:2	1.00 (ref)	0.75 (0.57, 1.00)	0.74 (0.54, 1.02)	0.87 (0.61, 1.24)	0.81 (0.53, 1.23)	0.43
<i>Trans</i> <i>cis</i> 18:2	1.00 (ref)	1.02 (0.77, 1.34)	1.02 (0.74, 1.39)	1.12 (0.80, 1.57)	1.15 (0.78, 1.70)	0.40
<i>Trans</i> <i>trans</i> 18:2	1.00 (ref)	1.17 (0.89, 1.54)	1.18 (0.89, 1.55)	1.25 (0.95, 1.66)	1.40 (1.05, 1.86)	0.02
Noncardiovascular disease mortality (n=1166)						
<i>Trans</i> 16:1n9	1.00 (ref)	0.91 (0.75, 1.11)	0.99 (0.80, 1.22)	0.83 (0.66, 1.05)	0.98 (0.76, 1.28)	0.86
Total <i>trans</i> 18:1 [†]	1.00 (ref)	0.90 (0.73, 1.11)	0.90 (0.71, 1.14)	1.03 (0.79, 1.35)	0.93 (0.68, 1.26)	0.91
<i>Cis</i> <i>trans</i> 18:2	1.00 (ref)	0.95 (0.77, 1.16)	0.93 (0.74, 1.17)	0.93 (0.72, 1.20)	1.00 (0.74, 1.34)	0.94
<i>Trans</i> <i>cis</i> 18:2	1.00 (ref)	0.94 (0.77, 1.15)	1.04 (0.83, 1.29)	1.11 (0.87, 1.41)	1.19 (0.91, 1.56)	0.09
<i>Trans</i> <i>trans</i> 18:2	1.00 (ref)	1.01 (0.83, 1.21)	1.04 (0.86, 1.25)	1.02 (0.85, 1.24)	1.16 (0.96, 1.42)	0.14

Models were adjusted for age (years), sex (male/female), race (white/nonwhite), education (<high school, high school, some college, college graduate), enrollment site, diabetes (yes/no), hypertension (yes/no), smoking (never/former/current), alcohol consumption (servings/week), physical activity (kcal/week), BMI (kg/m²), waist circumference (cm), dietary saturated fat (%energy), dietary monounsaturated fat (%energy), dietary fiber (g/day), plasma phospholipid EPA+DHA (% fatty acids), plasma phospholipid linoleic acid (% fatty acids), and other types of plasma phospholipid *trans*-fatty acids (% of fatty acids). BMI indicates body-mass index; EPA+DHA, eicosapentaenoic acids plus docosahexaenoic acids.

*P-trend was evaluated by assigning each participant the median value of each quintile category and evaluating this as a continuous variable.

[†]Total *trans*18:1 consisted of subtypes including *trans*-18:1n6, n7, n8, n9, n10-12 that were highly inter-related (pairwise correlations=0.83 to 0.93).

with wider confidence intervals and unchanged results of tests for trend (Figure S2).

In sensitivity analysis of *t/t*-18:2 without correction of laboratory drift, *t/t*-18:2 levels were positively associated with total mortality and each of the other outcomes except for non-CVD mortality (Figure 2). Magnitudes of these associations were larger compared with the original results correcting for laboratory drift. After excluding 285 participants in whom TFA levels were measured in 1999 (a subset enriched with early CHD cases), associations of *t/t*-18:2 with total mortality and CVD events were attenuated and no longer statistically significant in models including mutual adjustment for other TFAs (Figure 3). In contrast, even with these exclusions, *t/t*-18:2 remained associated with higher risk of total mortality and CVD outcomes in multivariable-adjusted models that were not mutually adjusted for all TFAs.

Discussion

In this large, prospective study of older Americans, we found divergent associations of different circulating TFA subtypes with total mortality, CVD mortality, and incident CHD. *t/t*-18:2 appeared most strongly associated with higher risk of total mortality, CVD mortality, and total CHD. *t/c*-18:2 was also positively associated with risk, but primarily only after mutual

adjustment for all TFAs. In contrast, *t*-16:1n9, *t*-18:1, and *c/t*-18:2 levels were not significantly associated with outcomes, particularly after accounting for reverse causation.

To our knowledge, no prior study has evaluated how different *t*-18:2 isomers relate to mortality or incident CHD. Prior studies, including from this cohort, have found that total *t*-18:2 relates to higher risk of fatal ischemic heart disease (IHD),¹⁸ sudden cardiac arrest,¹⁹ total⁴² and nonfatal CHD,²² possibly related to adverse lipid effects,⁴³ pro-inflammatory responses,^{9,10} and abnormal heart rate variability.⁴⁴ Our sensitivity analysis investigating total *t*-18:2 also found a positive association with total mortality. Yet, our isomer-specific analyses demonstrated substantial heterogeneity in associations of each *t*-18:2 isomers with mortality and CHD, with most pronounced adverse associations for *t/t*-18:2. Based on in vitro studies of human endothelial cells, *t/t*-18:2 has multiple adverse effects: increasing NF-κB, impairing endothelial insulin signaling and NO production, elevating superoxide production,⁴⁵ inducing apoptosis and necrosis in a dose- and time-dependent fashion.⁴⁶ Yet, these studies did not compare effects of other *t*-18:2 isomers. In addition, animal studies indicate that *t/t*-18:2 is preferentially incorporated into sn-1 position of phospholipids, similar to saturated fatty acids, whereas mono *t*-18:2 isomers such as *c/t*- or *t/c*-18:2 were acylated into sn-2 position, similar to linoleic acid.^{47,48} The fundamental biological differences may result in varying cardiovascular effects of *t*-18:2, as

Table 5. Prospective Association of Plasma Phospholipid *Trans*-Fatty Acid With Total Coronary Heart Disease With Mutual Adjustment (n=2742)

	Quintiles of Plasma Phospholipid <i>Trans</i> -Fatty Acid Levels					P Value For Trend*
	I	II	III	IV	V	
Total coronary heart disease (n=639)						
<i>Trans</i> 16:1n9	1.00 (ref)	1.26 (0.96, 1.64)	1.35 (1.01, 1.80)	1.36 (0.99, 1.87)	1.30 (0.90, 1.88)	0.22
Total <i>trans</i> 18:1 [†]	1.00 (ref)	0.89 (0.68, 1.18)	0.83 (0.61, 1.14)	0.78 (0.54, 1.13)	0.77 (0.51, 1.16)	0.23
<i>Cis</i> <i>trans</i> 18:2	1.00 (ref)	0.63 (0.48, 0.82)	0.57 (0.43, 0.77)	0.53 (0.38, 0.74)	0.50 (0.34, 0.73)	0.001
<i>Trans</i> <i>cis</i> 18:2	1.00 (ref)	1.13 (0.87, 1.48)	1.16 (0.86, 1.56)	1.65 (1.21, 2.25)	1.67 (1.17, 2.40)	0.002
<i>Trans</i> <i>trans</i> 18:2	1.00 (ref)	1.14 (0.88, 1.49)	1.43 (1.11, 1.85)	1.40 (1.07, 1.81)	1.39 (1.06, 1.83)	0.01
Coronary heart disease mortality (n=362)^{††}						
<i>Trans</i> 16:1n9	1.00 (ref)	1.28 (0.91, 1.81)	1.26 (0.86, 1.85)	1.20 (0.79, 1.83)	1.17 (0.71, 1.91)	0.68
Total <i>trans</i> 18:1 [†]	1.00 (ref)	0.70 (0.49, 1.00)	0.65 (0.43, 0.96)	0.60 (0.37, 0.98)	0.65 (0.38, 1.13)	0.20
<i>Cis</i> <i>trans</i> 18:2	1.00 (ref)	0.85 (0.61, 1.20)	0.75 (0.50, 1.11)	0.75 (0.48, 1.18)	0.64 (0.38, 1.08)	0.10
<i>Trans</i> <i>cis</i> 18:2	1.00 (ref)	0.92 (0.65, 1.31)	0.89 (0.60, 1.32)	1.32 (0.88, 1.98)	1.38 (0.86, 2.23)	0.07
<i>Trans</i> <i>trans</i> 18:2	1.00 (ref)	1.23 (0.87, 1.74)	1.30 (0.91, 1.84)	1.36 (0.96, 1.93)	1.36 (0.94, 1.96)	0.11
Nonfatal coronary heart disease (n=377)^{††}						
<i>Trans</i> 16:1n9	1.00 (ref)	1.36 (0.94, 1.96)	1.72 (1.17, 2.54)	1.61 (1.05, 2.47)	1.68 (1.03, 2.73)	0.06
Total <i>trans</i> 18:1 [†]	1.00 (ref)	1.09 (0.74, 1.59)	1.15 (0.76, 1.74)	1.00 (0.61, 1.61)	0.97 (0.56, 1.66)	0.72
<i>Cis</i> <i>trans</i> 18:2	1.00 (ref)	0.47 (0.33, 0.66)	0.44 (0.30, 0.64)	0.38 (0.25, 0.58)	0.35 (0.21, 0.56)	<0.001
<i>Trans</i> <i>cis</i> 18:2	1.00 (ref)	1.37 (0.96, 1.95)	1.52 (1.03, 2.24)	2.11 (1.41, 3.16)	2.06 (1.29, 3.28)	0.002
<i>Trans</i> <i>trans</i> 18:2	1.00 (ref)	1.10 (0.77, 1.56)	1.40 (1.00, 1.95)	1.29 (0.92, 1.82)	1.36 (0.95, 1.95)	0.08

Models were adjusted for age (years), sex (male/female), race (white/nonwhite), education (<high school, high school, some college, college graduate), enrollment site, diabetes (yes/no), hypertension (yes/no), smoking (never/former/current), alcohol consumption (servings/week), physical activity (kcal/week), BMI (kg/m²), waist circumference (cm), dietary saturated fat (%energy), dietary monounsaturated fat (%energy), dietary fiber (g/day), plasma phospholipid EPA+DHA (% fatty acids), plasma phospholipid linoleic acid (% fatty acids), and other types of plasma phospholipid *trans* fatty acids (% of fatty acids). BMI indicates body-mass index; EPA+DHA, eicosapentaenoic acids plus docosahexaenoic acids.

* P-trend was evaluated by assigning each participant the median value of each quintile category and evaluating this as a continuous variable.

[†]Total *trans*18:1 consisted of subtypes including *trans*-18:1n6, n7, n8, n9, n10-12 that were highly inter-related (pairwise correlations=0.83 to 0.93).

^{††}Subtypes of total coronary heart disease.

suggested in our study. Together with our findings, these experimental data support adverse cardiovascular effects of *t/t*-18:2.

t/c-18:2 was positively associated with total and nonfatal CHD after mutual adjustment for other TFAs. Because various TFA subtypes and isomers (in particular *t/c*- and *c/t*-18:2) may be causally intercorrelated due to common dietary sources, the findings following mutual adjustment should be interpreted cautiously. For example, our prior work in CHS showed that fried and bakery foods are common food sources of both *c/t*- and *t/c*-18:2.¹⁷ Whether the significant associations of *t/c*-18:2 with CHD represent a true effect or an artifact of overadjustment in this cohort is not known. Of note, combined levels of *t/c*- and *c/t*-18:2 have been shown to be associated with sudden cardiac arrest in this cohort.¹⁹

Unexpectedly, we observed *c/t*-18:2 to be inversely associated with incidence of total and nonfatal CHD. Yet, these inverse associations were attenuated and no longer statistically significant after we excluded cases occurring in

the first 2 years. Because the excluded cases were not younger than participants having events later in the follow-up, this result could not be interpreted as a stronger association of *c/t*-18:2 with premature CHD events. Instead, the findings suggested the possibility of reverse causation (ie, participants with substantial undiagnosed disease might have adapted healthier dietary habits or such disease could alter phospholipid *c/t*-18:2 levels). However, excluding earlier cases also hindered our ability to incorporate the short-term effects of *c/t*-18:2 on CHD incidence into the final estimates, which could partially explain the attenuation of the association. Our interpretation of the findings after excluding earlier cases could be speculative, and additional investigation of the biologic effects of *c/t*-18:2 are needed to confirm our findings.

Neither total *t*-18:1 nor *t*-16:1n9 were significantly associated with total mortality, CVD mortality, or CHD. These findings are consistent with prior biomarker studies showing no significant associations of erythrocyte membrane or

Table 6. Prospective Association of Plasma Phospholipid *Trans*-Fatty Acid With Cardiovascular and Noncardiovascular Mortality Without Mutual Adjustment (n=2742)

	Quintiles of Plasma Phospholipid <i>Trans</i> -Fatty Acid Levels					P Value For Trend*
	I	II	III	IV	V	
Cardiovascular mortality (n=567)						
<i>Trans</i> 16:1n9	1.00 (ref)	1.22 (0.94, 1.59)	1.01 (0.77, 1.32)	0.94 (0.71, 1.25)	0.98 (0.74, 1.30)	0.36
Total <i>trans</i> 18:1 [†]	1.00 (ref)	0.75 (0.58, 0.97)	0.67 (0.51, 0.87)	0.75 (0.57, 0.99)	0.73 (0.55, 0.96)	0.08
<i>Cis</i> <i>trans</i> 18:2	1.00 (ref)	0.70 (0.54, 0.90)	0.66 (0.50, 0.86)	0.78 (0.60, 1.01)	0.75 (0.56, 0.98)	0.12
<i>Trans</i> <i>cis</i> 18:2	1.00 (ref)	0.86 (0.67, 1.11)	0.81 (0.62, 1.06)	0.91 (0.70, 1.19)	0.90 (0.69, 1.18)	0.74
<i>Trans</i> <i>trans</i> 18:2	1.00 (ref)	1.14 (0.87, 1.50)	1.15 (0.88, 1.51)	1.22 (0.93, 1.60)	1.35 (1.02, 1.77)	0.03
Noncardiovascular disease mortality (n=1166)						
<i>Trans</i> 16:1n9	1.00 (ref)	0.91 (0.75, 1.09)	0.98 (0.82, 1.18)	0.86 (0.71, 1.05)	1.05 (0.87, 1.26)	0.62
Total <i>trans</i> 18:1 [†]	1.00 (ref)	0.89 (0.73, 1.07)	0.89 (0.74, 1.08)	1.04 (0.86, 1.26)	1.01 (0.83, 1.24)	0.36
<i>Cis</i> <i>trans</i> 18:2	1.00 (ref)	0.92 (0.77, 1.12)	0.95 (0.78, 1.14)	0.99 (0.82, 1.20)	1.13 (0.93, 1.38)	0.12
<i>Trans</i> <i>cis</i> 18:2	1.00 (ref)	0.91 (0.75, 1.09)	0.98 (0.81, 1.18)	1.06 (0.88, 1.28)	1.17 (0.97, 1.41)	0.02
<i>Trans</i> <i>trans</i> 18:2	1.00 (ref)	0.99 (0.82, 1.19)	1.03 (0.86, 1.24)	1.03 (0.86, 1.24)	1.16 (0.96, 1.40)	0.12

Models were adjusted for age (years), sex (male/female), race (white/nonwhite), education (<high school, high school, some college, college graduate), enrollment site, diabetes (yes/no), hypertension (yes/no), smoking (never/former/current), alcohol consumption (servings/week), physical activity (kcal/week), BMI (kg/m²), waist circumference (cm), dietary saturated fat (%energy), dietary monounsaturated fat (%energy), dietary fiber (g/day), plasma phospholipid EPA+DHA (% fatty acids), plasma phospholipid linoleic acid (% fatty acids). BMI indicates body-mass index; EPA+DHA, eicosapentaenoic acids plus docosahexaenoic acids.

*P-trend was evaluated by assigning each participant the median value of each quintile category and evaluating this as a continuous variable.

[†]Total *trans*18:1 consisted of subtypes including *trans*-18:1n6, n7, n8, n9, n10-12 that were highly interrelated (pairwise correlations=0.83 to 0.93).

adipose *t*-18:1 levels with risk of sudden cardiac arrest or nonfatal CHD.^{19,20,22} In a prior nested case-control study in CHS, we found an inverse association between plasma phospholipid *t*-18:1 and fatal CHD;¹⁸ this was no longer significant in this present analysis with more cases and longer follow-up time. Animal experiments suggest different *t*-18:1 isomers could have opposing influences on risk;^{45,49-52} therefore, effects may be masked when their sums are evaluated. Yet, *t*-18:1 isomers were highly intercorrelated in our cohort, precluding their separate evaluation.

The absence of association between circulating *t*-18:1 and CVD outcomes is puzzling, given that this fatty acid is the major contributor to total TFA consumption from PHVO, which has been consistently linked to adverse lipid effects in trials,^{4,53} and elevated CHD risk when estimated from self-reported dietary questionnaires.⁵³ This lack of association could be attributable, at least partly, to a survival effect, in that this cohort included elderly individuals who may already have survived higher exposure to TFA due to other characteristics protecting them from adverse outcomes. In addition, although *t*-18:1 is the major component of total TFA from PHVO, the correlation between circulating and dietary *t*-18:1 was low ($r=0.16$) in this cohort, consistent with the not high correlation between plasma and dietary TFAs in the Nurses' Health Study ($r=0.30$).⁵⁴ Circulating *t*-18:1 levels reflect not only the dietary intake, but also the yet unknown incorpora-

tion and metabolic processes that may result in different associations for circulating versus dietary TFAs. On the other hand, blood levels of dietary fatty acids generally provide more reliable estimates than self-reported intakes. The overall low laboratory coefficients of variation and the reasonable reliability of the exposures indicate the overall good measurements of circulating TFAs, despite the difficulty in quantifying TFAs with small peaks. Our results highlight the need to further investigate the determinants of *t*-18:1 biomarker levels and to confirm their relations to disease risk and the related mechanisms.

Our study has important public health implications. We identified positive associations of *t/c*- and *t/t*-18:2 with mortality and CVD. These results provide support for the recent US Food and Drug Administration proposal to remove PHVO from the list of additives that are generally recognized as safe.¹ In addition, the specific associations of these *t*-18:2 isomers with risks call attention to potential additional priorities for food policy. The recent US Food and Drug Administration proposal,¹ current food labeling laws,⁵⁵ and state and national regulations^{56,57} are all focused on PHVO and based on total TFA, which is predominantly *t*-18:1 and *t*-16:1n9. Yet, *c/t*- and *t/c*-18:2 can be produced via processes unrelated to partial hydrogenation, such as vegetable oil deodorization and high-temperature frying.⁵⁸⁻⁶⁰ If *t*-18:2 isomers are especially harmful for CVD, even at low

Table 7. Prospective Association of Plasma Phospholipid *Trans*-Fatty Acid With Total Coronary Heart Disease Without Mutual Adjustment (n=2742)

	Quintiles of Plasma Phospholipid <i>Trans</i> -Fatty Acid Levels					P Value For Trend*
	I	II	III	IV	V	
Total coronary heart disease (n=639)						
<i>Trans</i> 16:1n9	1.00 (ref)	1.17 (0.91, 1.51)	1.24 (0.96, 1.60)	1.18 (0.90, 1.53)	1.06 (0.80, 1.39)	0.92
Total <i>trans</i> 18:1 [†]	1.00 (ref)	0.95 (0.74, 1.22)	0.89 (0.69, 1.15)	0.87 (0.66, 1.13)	0.87 (0.67, 1.15)	0.29
<i>Cis</i> <i>trans</i> 18:2	1.00 (ref)	0.65 (0.51, 0.83)	0.64 (0.50, 0.82)	0.66 (0.51, 0.84)	0.67 (0.52, 0.87)	0.01
<i>Trans</i> <i>cis</i> 18:2	1.00 (ref)	0.90 (0.70, 1.15)	0.83 (0.64, 1.07)	1.08 (0.84, 1.38)	1.01 (0.79, 1.30)	0.45
<i>Trans</i> <i>trans</i> 18:2	1.00 (ref)	1.17 (0.90, 1.52)	1.46 (1.14, 1.88)	1.51 (1.17, 1.95)	1.48 (1.13, 1.93)	<0.001
Coronary heart disease mortality (n=362)^{††}						
<i>Trans</i> 16:1n9	1.00 (ref)	1.12 (0.80, 1.56)	1.03 (0.73, 1.44)	0.93 (0.65, 1.32)	0.89 (0.62, 1.28)	0.29
Total <i>trans</i> 18:1 [†]	1.00 (ref)	0.73 (0.53, 1.01)	0.69 (0.49, 0.95)	0.64 (0.45, 0.91)	0.69 (0.49, 0.99)	0.06
<i>Cis</i> <i>trans</i> 18:2	1.00 (ref)	0.76 (0.55, 1.03)	0.65 (0.47, 0.91)	0.72 (0.52, 1.00)	0.68 (0.48, 0.97)	0.04
<i>Trans</i> <i>cis</i> 18:2	1.00 (ref)	0.77 (0.56, 1.06)	0.67 (0.48, 0.95)	0.94 (0.68, 1.29)	0.87 (0.62, 1.21)	0.92
<i>Trans</i> <i>trans</i> 18:2	1.00 (ref)	1.20 (0.85, 1.69)	1.23 (0.88, 1.74)	1.36 (0.97, 1.90)	1.33 (0.93, 1.89)	0.10
Nonfatal coronary heart disease (n=377)^{††}						
<i>Trans</i> 16:1n9	1.00 (ref)	1.37 (0.96, 1.95)	1.78 (1.26, 2.51)	1.59 (1.11, 2.27)	1.46 (1.01, 2.12)	0.09
Total <i>trans</i> 18:1 [†]	1.00 (ref)	1.20 (0.86, 1.68)	1.30 (0.93, 1.82)	1.18 (0.82, 1.69)	1.20 (0.83, 1.74)	0.50
<i>Cis</i> <i>trans</i> 18:2	1.00 (ref)	0.57 (0.41, 0.79)	0.64 (0.47, 0.88)	0.63 (0.46, 0.87)	0.64 (0.46, 0.90)	0.03
<i>Trans</i> <i>cis</i> 18:2	1.00 (ref)	1.06 (0.76, 1.47)	1.05 (0.75, 1.46)	1.30 (0.94, 1.80)	1.18 (0.84, 1.66)	0.20
<i>Trans</i> <i>trans</i> 18:2	1.00 (ref)	1.16 (0.82, 1.65)	1.56 (1.12, 2.17)	1.55 (1.11, 2.17)	1.58 (1.12, 2.24)	<0.001

Models were adjusted for age (years), sex (male/female), race (white/nonwhite), education (<high school, high school, some college, college graduate), enrollment site, diabetes (yes/no), hypertension (yes/no), smoking (never/former/current), alcohol consumption (servings/week), physical activity (kcal/week), BMI (kg/m²), waist circumference (cm), dietary saturated fat (%energy), dietary monounsaturated fat (%energy), dietary fiber (g/day), plasma phospholipid EPA+DHA (% fatty acids), plasma phospholipid linoleic acid (% fatty acids). BMI indicates body-mass index; EPA+DHA, eicosapentaenoic acids plus docosahexaenoic acids.

*P-trend was evaluated by assigning each participant the median value of each quintile category and evaluating this as a continuous variable.

[†]Total *trans*18:1 consisted of subtypes including *trans*-18:1n6, n7, n8, n9, n10-12 that were highly inter-related (pairwise correlations=0.83 to 0.93).

^{††}Subtypes of total coronary heart disease.

exposure levels such as seen in our study, then current policy measures may not fully capture all major sources of these fatty acids. On the global level, while a limited number of countries such as Denmark,⁵⁷ Argentina,⁶¹ and the United States¹ have national regulations limiting TFAs, the majority of the world does not. Many countries such as Mexico,⁶² Egypt,⁶³ and Iran⁵⁹ still have high consumption of TFAs, with little active proposed regulation. Other countries such as India have only proposed but not enforced regulation.⁶⁴ Our findings provide useful insights to inform policy priorities not only in the United States, but also in these additional countries. In addition to policy implication, our findings inform scientific knowledge of how different TFA subtypes may influence total and cause-specific mortality, an area that is not well understood and with many remaining questions. Our results also highlight the need for further investigation of dietary determinants, other drivers, and independent disease, physiologic, and molecular effects of *t*-18:2 isomers and other circulating TFA subtypes.

Our study has several strengths. We utilized a well-established prospective cohort with little loss to follow-up, minimizing selection bias. TFA levels were assessed using biomarkers, providing objective measures of exposure and allowing assessment of individual TFA subtypes and isomers. Careful follow-up and adjudication of events minimized the potential for missed or misclassified outcomes, and the large number of cases increased statistical power to detect associations. Information on other risk factors was prospectively collected using standard methods, increasing our ability to adjust for confounding. We performed several sensitivity analyses to evaluate the robustness of each finding.

Potential limitations deserve consideration. TFA levels were measured at baseline, and changes in exposure over time would result in misclassification and may attenuate true associations. We partly accounted for this using regression dilution correction, but this may incompletely account for within-person variation over time. Laboratory drift was

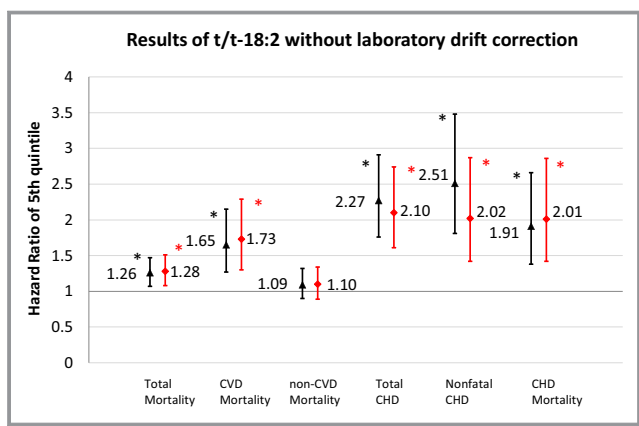


Figure 2. Prospective associations of plasma phospholipid *trans/trans*-18:2 with total mortality, CVD and CHD before and after mutual adjustment without correction of laboratory drift. Black triangles indicate multivariate-adjusted hazard ratios comparing adults in the top quintile category to adults in the bottom quintile category for *t/t*-18:2 fatty acid levels, before mutual adjustment for the 5 TFAs and without correction of laboratory drift; red diamonds, after mutual adjustment for the 5 TFAs and without correction of laboratory drift. Error bars represent 95% CI. All risk estimates were adjusted for age, sex, race, education, enrollment site, diabetes, hypertension, smoking, alcohol consumption, physical activity, BMI, waist circumference, dietary saturated fat, dietary monounsaturated fat, dietary fiber, plasma phospholipid EPA+DHA, plasma phospholipid linoleic acid. **P*-trend<0.05 across quintiles. BMI indicates body-mass index; CHD, coronary heart disease; CVD, cardiovascular disease; EPA+DHA, EPA+DHA, eicosapentaenoic acids plus docosahexaenoic acids; TFA, *trans*-fatty acid.

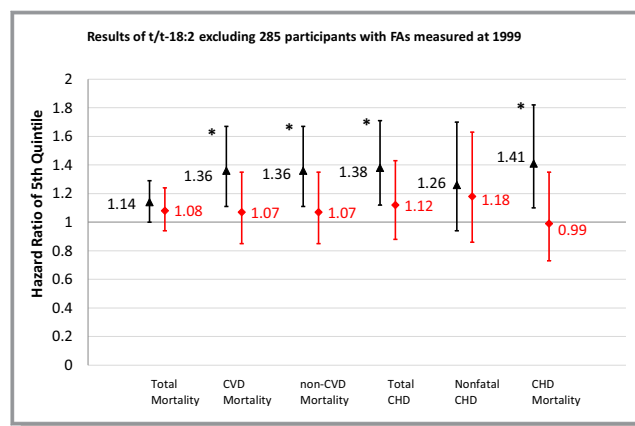


Figure 3. Prospective associations of plasma phospholipid *trans/trans*-18:2 with total mortality, CVD, and CHD before and after mutual adjustment after excluding 285 participants with *trans/trans*-18:2 levels measured in 1999. Black triangles indicate multivariate-adjusted hazard ratios comparing adults in the top quintile category to adults in the bottom quintile category for *t/t*-18:2 fatty acid levels, before mutual adjustment for the 5 TFAs and excluding 285 participants with *t/t*-18:2 measured early; red diamonds, after mutual adjustment for the 5 TFAs and excluding 285 participants with *t/t*-18:2 measured early. Error bars represent 95% CI. All risk estimates were adjusted for age, sex, race, education, enrollment site, diabetes, hypertension, smoking, alcohol consumption, physical activity, BMI, waist circumference, dietary saturated fat, dietary monounsaturated fat, dietary fiber, plasma phospholipid EPA+DHA, plasma phospholipid linoleic acid. **P*-trend<0.05 across quintiles. BMI indicates body-mass index; CHD, coronary heart disease; CVD, cardiovascular disease; EPA+DHA, eicosapentaenoic acids plus docosahexaenoic acids; TFA, *trans*-fatty acid.

observed for *t/t*-18:2, possibly related to reduced assay stability for fatty acids with very low concentrations. In sensitivity analyses excluding participants with fatty acids measured in 1999, *t/t*-18:2 was no longer significantly associated with mortality or CHD after mutual adjustment for all TFAs, but remained significantly positively associated prior to such mutual adjustment, which would be unrelated to laboratory drift. In addition, our main analyses corrected for laboratory drift using calibration, decreasing its influence on our findings. The cohort comprised older, largely white Americans, and survival bias could partly explain the absence of associations for *t*-18:1 and *t*-16:1n9. Although the great majority of CVD occurs after age 65, which is the minimum enrollment age in CHS, the associations of TFA subtypes may differ in a much younger population.

In conclusion, our findings suggest that later in life, circulating *t/t*-18:2 levels are associated with higher risk of total mortality, mainly due to increased CVD mortality and higher risk of total CHD; and *t/c*-18:2 with higher total mortality and total CHD. These findings highlight the potentially varying health effects of different TFAs, with implications for both future research and for designing and

evaluating measures to reduce population risk due to TFA exposures.

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