



# Circulating and Dietary *Trans* Fatty Acids and Incident Type 2 Diabetes in Older Adults: The Cardiovascular Health Study

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## OBJECTIVE

To investigate the effects of *trans* fatty acids (TFAs) on type 2 diabetes mellitus (DM) by specific TFA subtype or method of assessment.

## RESEARCH DESIGN AND METHODS

In the Cardiovascular Health Study, plasma phospholipid *trans* (t)-16:1n9, t-18:1, and *cis* (c)/t-, t/c-, and t/t-18:2 were measured in blood drawn from 2,919 adults aged 74 ± 5 years and free of prevalent DM in 1992. Dietary TFA was estimated among 4,207 adults free of prevalent DM when dietary questionnaires were initially administered in 1989 or 1996. Incident DM was defined through 2010 by medication use or blood glucose levels. Risks were assessed by Cox proportional hazards.

## RESULTS

In biomarker analyses, 287 DM cases occurred during 30,825 person-years. Both t-16:1n9 (extreme quartile hazard ratio 1.59 [95% CI 1.04–2.42], *P*-trend = 0.04) and t-18:1 (1.91 [1.20–3.03], *P*-trend = 0.01) levels were associated with higher incident DM after adjustment for de novo lipogenesis fatty acids. In dietary analyses, 407 DM cases occurred during 50,105 person-years. Incident DM was positively associated with consumption of total TFAs (1.38 [1.03–1.86], *P*-trend = 0.02), t-18:1 (1.32 [1.00–1.76], *P*-trend = 0.04), and t-18:2 (1.41 [1.05–1.89], *P*-trend = 0.02). After further adjustment for other dietary habits, however, the associations of estimated dietary TFA with DM were attenuated, and only nonsignificant positive trends remained.

## CONCLUSIONS

Among older adults, plasma phospholipid t-16:1n9 and t-18:1 levels were positively related to DM after adjustment for de novo lipogenesis fatty acids. Estimated dietary TFA was not significantly associated with DM. These findings highlight the need for further observational, interventional, and experimental studies of the effects TFA on DM.

Substantial evidence has linked consumption of *trans* fatty acids (TFAs), unsaturated fatty acids with at least one double bond in the *trans* configuration, to an increased risk of coronary heart disease (CHD) (1–3). However, the effects of TFA on type 2 diabetes mellitus (DM) remain unclear. In some animal models (4,5) but not others (6,7), the feeding of TFA reduced insulin sensitivity and glucose uptake by altering

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the adipocyte plasma membrane fatty acid composition and fluidity (4) and/or by changing the gene expression of several proteins related to insulin sensitivity (5). In other experimental studies, higher TFA levels increased hepatic de novo lipogenesis, leading to nonalcoholic steatohepatitis and insulin resistance (8–10). Short-term trials in humans have shown mixed results. Among healthy adults fed TFAs, no significant effects on glucose and insulin metabolism were seen (11–13), whereas among obese adults with prevalent diabetes or hyperlipidemia, TFA diets produced deleterious effects on glucose-insulin homeostasis (14,15). Overall, these findings suggest that TFA could increase DM risk, especially among participants predisposed to insulin resistance, although the generalizability of the findings of experimental studies and trials to long-term effects of usual TFA consumption remains unclear.

Only a few observational studies have assessed long-term dietary TFA and incident DM, with mixed results (16–18). Most of these previous studies evaluated estimated total TFA intake but not TFA subtypes that vary by length of the fatty acid chain and by number and location of the *trans* double bonds. Several studies of CHD suggested that individual TFA subtypes may have varying effects on risk (3,19,20), yet potential effects of various TFA subtypes on incident DM are largely unknown. Two prospective studies found an inverse association between phospholipid *trans* (*t*)-16:1n7, a naturally occurring TFA in dairy products, and incident DM (21,22), but these studies did not evaluate other TFAs. Little is known about how other TFA subtypes influence DM.

Additionally, few prior studies evaluated both dietary and circulating TFA, which have different advantages and constraints. TFA consumption estimated from dietary questionnaires represents long-term intake and may be limited by substantial measurement error and a reduced ability to quantify TFA subtypes. Conversely, TFA biomarkers provide objective estimates of tissue exposure to specific TFA subtypes and incorporate the influences of both diet and relevant biological processes but reflect a shorter period of exposure (weeks to months). Thus, both dietary estimates and biomarker levels of TFA

provide important measures of exposure with complementary strengths and limitations. To elucidate the potential effects of TFAs on DM, our primary aim was to investigate the prospective associations of plasma phospholipid TFA subtypes, including *t*-16:1n9, *t*-18:1, and *cis* (*c*)/*t*-, *t*/*c*-, and *t*/*t*-18:2, as well as to secondarily evaluate estimated dietary TFAs, including total TFA, *t*-18:1, and *t*-18:2, with incident DM.

## RESEARCH DESIGN AND METHODS

### Design and Population

The Cardiovascular Health Study (CHS) is a community-based, multicenter, prospective cohort of older U.S. adults (23). Briefly, 5,201 ambulatory, noninstitutionalized adults aged  $\geq 65$  years were randomly enrolled in 1989–90 from Medicare eligibility lists in four U.S. communities (Forsyth County, NC; Sacramento County, CA; Washington County, MD; Allegheny County, PA); an additional 687 minority participants were similarly enrolled in 1992–1993. Among all eligible adults, 57% agreed to participate. The institutional review committee from each center approved the study, and all participants provided informed written consent.

Plasma phospholipid fatty acids were measured in 3,941 participants with available blood samples taken in 1992–93. In biomarker analyses, after excluding 912 participants with prevalent DM in 1992–93 and 110 with missing follow-up information on DM diagnosis, we included 2,919 participants as the study population. Dietary TFA was estimated from dietary questionnaires administered in 1989–1990 ( $n = 5,179$ ) and 1995–1996 ( $n = 3,797$ ), including 5,673 total participants completing at least one questionnaire. In dietary analyses, after excluding 1,328 individuals with prevalent DM at the time of initial dietary assessment and 138 with missing follow-up information to DM diagnosis, we included 4,207 participants as the study population.

### Plasma Phospholipid TFA

Plasma phospholipid fatty acid composition was measured at the Fred Hutchinson Cancer Research Center (Supplementary Data). Total lipids were extracted from plasma, and the phospholipid fraction was isolated by one-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 *t*-16:1n9, *t*-16:1n7, *t*-18:1n6 to *t*-18:6n12, and *c*/*t*-, *t*/*c*-, and *t*/*t*-18:2 isomers. All *t*-18:1 isomers ( $r > 0.83$ ) were summed to evaluate total *t*-18:1 because of their high intercorrelation. Laboratory coefficients of variance were 5% for *t*-16:1n9, 3% for *t*-16:1n7, 2% for total *t*-18:1, and 8% for total *t*-18:2.

Blood drawn and stored in 1992 was used for fatty acid measurements, which were performed in the same laboratory using similar methods but across two time periods as follows: 493 samples in 1999–2002 and 3,884 in 2007–2012. Potential between-period variation was evaluated and corrected by means of regression analysis using duplicate measures in both time periods of the same blood samples from 163 CHS participants (20). To assess long-term reliability, circulating TFAs were measured serially in 100 CHS participants using different blood samples drawn in 1992–1993 and 2005–2006. The baseline and 13-year intercorrelation was 0.37 for *t*-16:1n9, 0.60 for *t*-18:1, 0.42 for *c*/*t*-18:2, 0.18 for *t*/*c*-18:2, and 0.46 for *t*/*t*-18:2. These intercorrelations are comparable to long-term reliability of other physiologic risk factors, such as blood pressure and blood cholesterol levels (24,25), except for *t*/*c*-18:2 for which lower reliability was seen. In the current study, plasma phospholipid TFA levels were measured as the percentage of total fatty acids.

### Dietary TFA

Usual dietary habits were assessed using semiquantitative food frequency questionnaires in 1989–1990 and 1995–1996. The 1989–1990 questionnaire, a 99-item, picture sort, interviewer-administered survey, included five responses (26). The 1995–1996 questionnaire, a 131-item self-administered survey, included nine responses (27). Both questionnaires were valid and reliable compared with repeated 24-h dietary recalls or 1-week dietary records (26,27). To obtain an estimate of total or subtype dietary TFAs, the frequency response for each food item was multiplied by the estimated contemporaneous TFA content of that food based on the Harvard Nutrient Database (28) for its standardized serving

size, and the amounts were summed across all foods. We cumulatively updated dietary TFA intake among participants who completed both the 1989–1990 and the 1995–1996 questionnaires ( $n = 3,894$ ,  $r = 0.40$  for repeats of total TFA intake), and for participants enrolled in 1992–1993 ( $n = 313$ ), we used TFA consumption as estimated from the 1995–1996 questionnaire, which was considered the baseline year at risk for these participants.

#### Ascertainment of Events

Participants brought in and reported all prescription medication taken in the previous 2 weeks during the annual study examination through 1999; similar information was collected annually thereafter by telephone. Medication information was complete for 96.4% of person-time through 2010. DM cases were defined by new use of insulin or hypoglycemic medication, fasting glucose  $\geq 126$  mg/dL (assessed in 1989, 1992, 1996, 1998, and 2005), nonfasting glucose  $\geq 200$  mg/dL (assessed in 1994), or 2-h postchallenge glucose (oral glucose tolerance test [OGTT])  $\geq 200$  mg/dL (assessed in 1989 and 1996).

Traditional risk factors for DM had varying relationships with incident DM, depending on preceding degrees of insulin resistance or pancreatic  $\beta$ -cell dysfunction before diagnosis (29). In exploratory analyses, we subclassified incident DM cases into those with preceding higher insulin resistance, lower  $\beta$ -cell function, or both as estimated by HOMA for insulin resistance (HOMA-IR) and  $\beta$ -cell function (HOMA-B).

#### Covariates

Information on sociodemographic, lifestyle, and clinical risk factors was collected at annual clinic visits (23). Cardiovascular disease (CVD), including CHD, congestive heart failure, atrial fibrillation, and stroke, was diagnosed and reviewed by centralized adjudication committees. Fasting total cholesterol, HDL cholesterol, and triglyceride levels were measured using blood samples, and LDL cholesterol was calculated using the Friedewald equation among individuals without hypertriglyceridemia (30). For all biomarker and dietary analyses, we used covariates measured at the same study visit as the exposure assessment.

#### Statistical Analysis

TFA levels were evaluated in quartiles as categorical variables. To test for trend, we assigned each participant a median value of the corresponding quartile and evaluated the variable continuously. Cox proportional hazards were used to examine longitudinal associations of TFAs with incident DM, with follow-up time through 2010 as the time metric. The proportional hazard assumption was not rejected on the basis of Schoenfeld residuals. Missing covariates (most factors  $< 2\%$ , dietary factors 4–10%) were imputed by best subsets regression. For biomarker analyses, we adjusted for age, sex, race, education, enrollment site, BMI, waist circumference, smoking status, alcohol intake, physical activity, CVD, hypertension, and plasma phospholipid levels of 16:0 and 18:0. For dietary analyses, we adjusted for similar demographics, lifestyle, and clinical risk factors as in the biomarker analyses, with additional adjustment for dietary habits, including consumption of coffee, red meat, glycemic load, fiber, polyunsaturated fat, saturated fat, and total energy. Instead of individually adjusting for dietary habits, we adjusted for a previously established dietary score in CHS calculated based on consumption of whole grains, fish, fruits and vegetables, nuts and seeds, red and processed meat, sugar-sweetened beverages, and fried potatoes (31). Because associations of ruminant-derived  $t$ -16:1n7 with DM in CHS have been previously reported but only with follow-up through 2006 (21), in secondary analyses, we investigated the associations of circulating  $t$ -16:1n7 and dietary  $t$ -16:1 with incident DM.

In exploratory analyses, we adjusted for metabolic risk factors, including HDL cholesterol, triglyceride, blood insulin, and glucose levels, to evaluate potential for mediation. To assess associations of circulating and dietary TFA with DM cases of potentially differing etiology, we stratified the entire population at risk by levels of HOMA-IR and HOMA-B in 1992–1993 (29). We also explored modification effects of baseline age, sex, race, HOMA-IR, waist circumference, total/HDL cholesterol ratio, and physical activity using multiplicative interaction terms.

In sensitivity analyses, we evaluated incident DM based on only medication use and fasting glucose levels (i.e.,

excluding data on OGTT levels). Due to shared dietary sources and modest intercorrelations of each phospholipid TFA subtype (32), we simultaneously adjusted for the five circulating TFAs to explore independent associations. We censored the cohort at the midpoint of follow-up to minimize misclassification due to exposure variation over time and excluded events within the first 2 years to minimize reverse causation from preexisting subclinical disease. We also evaluated associations of circulating total  $t$ -18:2. Possible linear and nonlinear associations were evaluated using multivariable-adjusted restricted cubic splines. All analyses were performed using Stata 11 (StataCorp, College Station, TX) with two-tailed  $\alpha = 0.05$ , except for exploratory analyses of DM subclasses and effect modification for which we conducted Bonferroni correction with two-tailed  $\alpha = 0.002$  and 0.001, respectively.

#### RESULTS

At baseline, mean  $\pm$  SD age was  $74 \pm 5.3$  years, and 61% of participants were female. Concentrations of circulating TFAs varied from  $2.01 \pm 0.73\%$  of fatty acids for  $t$ -18:1 to  $0.05 \pm 0.02$  for  $t$ -18:2 (Table 1). Circulating TFA subtypes were at most modestly intercorrelated. In addition, partial correlations between circulating and dietary TFAs were very low ( $r = -0.1$  to  $0.1$ ), possibly being related to the 3-year time period between measurements, measurement error in the dietary questionnaires, differences in time periods represented by each exposure, or (unknown) metabolic influences on circulating TFA levels.

In unadjusted cross-sectional analyses at baseline, circulating TFA levels were higher in white participants, except for  $t$ -18:2, which was higher in black participants (Table 2). Each TFA subtype was associated with substantially lower alcohol intake and lower circulating 16:0 and 18:0 levels. Among metabolic risk factors,  $t$ -18:1 was inversely associated with BMI and waist circumference, and each of the circulating TFA subtypes was inversely associated with HDL cholesterol. In similar unadjusted cross-sectional analyses of estimated dietary TFA (Supplementary Table 1), total TFA,  $t$ -18:1, and  $t$ -18:2 were positively associated with waist circumference and consumption of

**Table 1—Concentration of plasma phospholipid and dietary TFAs and their partial correlations in the CHS**

	Plasma phospholipid TFAs (n = 2,919)					Dietary TFAs (n = 4,207)		
	t-16:1n9	t-18:1*	c/t-18:2	t/c-18:2	t/t-18:2	Total TFA	t-18:1	t-18:2
Mean (SD)†	0.07 (0.02)	2.01 (0.73)	0.08 (0.02)	0.13 (0.06)	0.05 (0.02)	3.68 (1.26)	2.03 (0.69)	0.53 (0.17)
5th, 95th percentile‡	0.04, 0.12	0.97, 3.27	0.04, 0.12	0.07, 0.23	0.03, 0.07	1.74, 5.95	0.95, 3.24	0.07, 0.27
Partial Pearson correlation‡	Plasma phospholipid TFAs					Dietary TFAs		
t-16:1n9 (cv = 0.05)	1.00	—	—	—	—	Total TFA	1.00	—
t-18:1* (cv = 0.02)	0.65	1.00	—	—	—	t-18:1	0.96	1.00
c/t-18:2 (cv = 0.08)	0.09	0.28	1.00	—	—	t-18:2	0.60	−0.36
t/c-18:2 (cv = 0.08)	−0.11	0.10	0.67	1.00	—	—	—	—
t/t-18:2 (cv = 0.08)	0.01	0.07	−0.13	0.09	1.00	—	—	—

Dietary TFAs were assessed at the year when participants completed their initial dietary questionnaire (1989–1990 for participants enrolled in 1989–1990; 1995–1996 for participants enrolled in 1992–1993). cv, coefficient of variation. \*Total t-18:1 comprised subtypes t-18:1n6, n7, n8, n9, n10, n11, and n12, with intercorrelation between each subtype ranging from 0.83 to 0.93. †Unit for plasma phospholipid TFA is % of fatty acid; unit for dietary TFA is g/day, with energy correction. ‡Partial Pearson correlation adjusted for age, sex, race, education, enrollment site, smoking status, alcohol consumption, physical activity, BMI, waist circumference, prevalence of CVD, and hypertension at baseline.

caffeinated coffee, red meat, and saturated and polyunsaturated fatty acids and inversely associated with education and consumption of decaffeinated coffee, fiber, and glycemic load.

In biomarker analyses, 287 new DM cases occurred during 30,825 person-years (Supplementary Fig. 1). After adjustment for demographic, lifestyle, and clinical risk factors, circulating t-16:1n9, t-18:1, and c/t-, t/c-, and t/t-18:2 were each not significantly associated with incident DM (Table 3). However, after further adjustment for plasma phospholipid 16:0 and 18:0, both t-16:1n9 and t-18:1 were positively associated with incident DM, whereas the t-18:2 isomers remained unassociated. Compared with the lowest quartile, participants in the highest quartile of t-16:1n9 and t-18:1 had a 59% and 91% increased risk of incident DM, respectively (*P*-trend = 0.04 and 0.01). After mutual adjustment for the five TFA subtypes, only t-18:1 remained positively related to incident DM (extreme quartile hazard ratio [HR] 1.95 [95% CI 1.18–3.21] data not shown). Further adjustment for circulating c-18:2, -14:0, -15:0, -17:0, -16:1n7, -16:1n9, -18:1n7, and -18:1n9; circulating t-16:1n7, eicosapentaenoic acid, docosahexaenoic acid, and  $\alpha$ -linolenic acid; use of statins; and metabolic risk factors, including baseline levels of HDL cholesterol, triglycerides, blood insulin, and glucose levels, did not appreciably alter the results (data not shown).

As previously reported (21), phospholipid t-16:1n7 was inversely associated with incident DM (HR 0.38 [95% CI 0.24–0.62], *P*-trend < 0.001), adjusting for demographic, clinical, and lifestyle

risk factors. After further adjustment for circulating 16:0 and 18:0, only a nonsignificant inverse trend was apparent (*P*-trend = 0.10) (Table 3).

In dietary analyses, total TFA, t-18:1, and t-18:2 but not t-16:1 were associated with a higher risk of incident DM after adjustment for demographic, lifestyle, and clinical risk factors (Table 4). Compared with the lowest quartile, participants in the highest quartile of total TFA, t-18:1, and t-18:2 had 38%, 32%, and 41% higher risk, respectively (*P*-trend = 0.02, 0.04, and 0.02, respectively). Further adjustment for individual dietary factors did not appreciably alter the magnitude of these risk estimates but widened the CIs, with resulting nonsignificant trends toward higher risk for total TFA and t-18:1 (*P*-trend = 0.06 and 0.09) and remaining significantly higher risk for t-18:2 (*P*-trend = 0.03). However, adjusting for a composite dietary score attenuated the associations for all dietary TFA subtypes, and none remained significantly related to incident DM.

In exploratory analyses stratified by HOMA-IR and HOMA-B levels, circulating TFA subtypes were not significantly associated with different subclasses of incident DM after Bonferroni correction (Supplementary Table 2), although a nonsignificant positive trend was seen between circulating t-18:1 and incident DM cases with both higher insulin resistance and lower  $\beta$ -cell function (*P*-trend = 0.004). In addition, dietary total TFA and t-18:1 appeared to be related to a higher risk of incident DM with both higher insulin resistance and lower  $\beta$ -cell function (*P*-trend  $\leq$  0.02 each),

although associations were nonsignificant with Bonferroni correction.

In sensitivity analyses, excluding events within the first 2 years of follow-up or censoring follow-up at 9 years had little effect on the results for both circulating and dietary TFAs. Defining DM cases without the use of OGTT levels had little effect on the results for circulating TFAs but substantially strengthened the positive association for each dietary TFA possibly due to reverse causation of the misclassified prevalent DM cases (Supplementary Table 3). Total t-18:2 levels were not significantly associated with incident DM. Multivariable-adjusted restricted cubic splines demonstrated significant positive linear associations for circulating t-16:1n9 (*P*-linearity = 0.02) and t-18:1 (*P*-linearity = 0.04), with little evidence for nonlinearity (*P*-nonlinearity > 0.60 each) (Supplementary Fig. 2). In addition, there was little evidence for effect modification by baseline age, sex, race, HOMA-IR, waist circumference, total/HDL cholesterol ratio, and physical activity (*P*-interactions  $\geq$  0.20 for each).

## CONCLUSIONS

In this large prospective study of older Americans, levels of plasma phospholipid t-16:1n9 and t-18:1 but not t-18:2 isomers were positively associated with incident DM only after adjustment for 16:0 and 18:0 levels. Estimated dietary total TFA, t-18:1, and t-18:2 but not t-16:1 were associated with a higher risk of DM in non-diet-adjusted models, but only nonsignificant positive trends remained after adjustment for a comprehensive dietary score.

**Table 2—Baseline characteristics of 2,919 participants by quartile of circulating TFA concentration**

	Quartile of t-161n9		Quartile of t-181		Quartile of c/t-182		Quartile of t/c-182		Quartile of t/c-182	
	I	IV	I	IV	I	IV	I	IV	I	IV
Total fat (%)	0.04 ± 0.01	0.11 ± 0.02	1.18 ± 0.23	2.98 ± 0.55	0.05 ± 0.01	0.11 ± 0.02	0.08 ± 0.01	0.21 ± 0.05	0.03 ± 0.004	0.07 ± 0.01
Range total fat (%)	0.02–0.05	0.09–0.25	0.23–1.48	2.43–8.46	0.01–0.06	0.09–0.26	0.03–0.10	0.16–0.73	0.01–0.04	0.06–0.14
Adults in quartile (n)	716	712	718	740	752	694	744	713	793	760
Age (years)	73.3 ± 4.7	74.9 ± 5.5*	73.7 ± 5.0	75.9 ± 5.5*	74.2 ± 5.4	74.1 ± 5.1	74.2 ± 5.5	74.4 ± 5.1	73.7 ± 5.1	74.6 ± 5.5*
Male sex	40	40	38	40	44	36*	42	39	34	53*
White race	87	92*	84	92*	77	97*	81	94*	95	95
High school education	49	44	54	39*	50	42*	51	44*	47	38*
CVD†	26	27	26	27	23	28*	28	28	25	29*
Hypertension†	45	44	45	39	44	44	45	43	43	47
Current smoker	9	9	9	11	9	9	9	11	10	10
Former smoker	48	41*	49	40*	47	42	48	44	45	44
Alcohol (drinks/week)	4.7 ± 10.8	0.9 ± 2.5*	4.8 ± 10.9	1.0 ± 3.7*	3.4 ± 10.0	1.4 ± 4.4*	3.0 ± 9.8	1.6 ± 4.5*	3.2 ± 9.9	1.8 ± 5.3*
Physical activity (kcal/week)	1,269 ± 1,750	956 ± 1,275*	1,154 ± 1,632	997 ± 1,307	1,147 ± 1,535	1,067 ± 1,473	1,192 ± 1,650	1,049 ± 1,449	1,077 ± 1,490	1,004 ± 1,290*
BMI (kg/m <sup>2</sup> )	26.6 ± 4.3	25.8 ± 4.3*	26.6 ± 4.4	25.6 ± 4.1*	26.5 ± 4.6	26.2 ± 4.3	26.2 ± 4.7	26.3 ± 4.4	27.0 ± 4.5	26.0 ± 4.4
Waist circumference (cm)	96.2 ± 12.7	94.5 ± 11.8	96.5 ± 12.8	94.1 ± 11.6*	95.9 ± 13.0	95.7 ± 12.4	95.3 ± 13.1	96.6 ± 13.0	97.6 ± 13.0	96.6 ± 12.2*
Triglycerides (mg/dL)	137 ± 86	135 ± 75	137 ± 80	136 ± 78	121 ± 65	151 ± 87*	122 ± 61	149 ± 83*	169 ± 87	208 ± 38
HDL cholesterol (mg/dL)	57.2 ± 16.1	52.6 ± 13.3*	58.0 ± 15.9	52.6 ± 13.9*	55.9 ± 14.5	53.7 ± 14.6*	55.8 ± 14.5	53.0 ± 14.0*	53.7 ± 14.6	51.1 ± 13.1*
Insulin (international units/mL)	11.6 ± 10.6	9.9 ± 4.8*	11.5 ± 10.5	10.0 ± 4.7	11.2 ± 15.6	10.6 ± 5.6	10.5 ± 6.2	10.9 ± 6.5	11.9 ± 10.1	11.3 ± 7.6*
Glucose (mg/dL)	98.2 ± 10.5	95.9 ± 8.5*	98.4 ± 10.5	95.9 ± 8.8*	98.0 ± 9.5	95.8 ± 9.0*	97.8 ± 9.7	96.7 ± 9.3	97.4 ± 10.2	97.5 ± 10.4
Plasma phospholipid biomarker										
16:0 fatty acid	26.2 ± 1.62	24.4 ± 1.27*	26.4 ± 1.59	24.1 ± 1.20*	25.5 ± 1.49	25.0 ± 1.61*	25.5 ± 1.52	25.0 ± 1.54*	26.0 ± 1.71	24.7 ± 1.39*
18:0 fatty acid	13.6 ± 1.22	13.2 ± 0.96*	13.4 ± 1.28	13.3 ± 1.00*	13.6 ± 1.08	13.2 ± 1.10*	13.5 ± 1.06	13.4 ± 1.09*	13.5 ± 1.25	13.4 ± 0.97*

Data are mean ± SD or % unless otherwise indicated. Baseline characteristics among adults in the lowest and highest quartiles for each TFA level are presented. \**P* < 0.01 for trend across quartiles. †CVD includes congestive heart failure, CHD, myocardial infarction, stroke, and atrial fibrillation. ‡Hypertension defined as a systolic and diastolic blood pressure ≥140/90 mmHg or use of antihypertensive treatment.

**Table 3—Prospective association of plasma phospholipid TFAs with incident diabetes (n = 2,919)**

	Quartile of plasma phospholipid TFA subtypes				P-trend value
	I	II	III	IV	
<b>t-16:1n9</b>					
Cases (person-years)	81 (7,598)	80 (8,207)	63 (7,903)	63 (7,118)	—
Multivariate*	1.00 (ref)	0.93 (0.68, 1.27)	0.79 (0.57, 1.11)	0.94 (0.67, 1.32)	0.57
Multivariate + DNL FAs†	1.00 (ref)	1.10 (0.79, 1.53)	1.10 (0.76, 1.60)	1.59 (1.04, 2.42)	0.04
<b>t-18:1</b>					
Cases (person-years)	75 (7,627)	83 (7,862)	64 (7,803)	65 (7,533)	—
Multivariate*	1.00 (ref)	1.07 (0.78, 1.48)	0.90 (0.63, 1.26)	0.97 (0.68, 1.37)	0.64
Multivariate + DNL FAs†	1.00 (ref)	1.33 (0.95, 1.87)	1.32 (0.89, 1.94)	1.91 (1.20, 3.03)	0.01
<b>c/t-18:2</b>					
Cases (person-years)	76 (7,950)	77 (8,222)	75 (7,705)	59 (6,948)	—
Multivariate*	1.00 (ref)	1.07 (0.77, 1.47)	1.09 (0.79, 1.52)	0.94 (0.66, 1.35)	0.78
Multivariate + DNL FAs†	1.00 (ref)	1.09 (0.79, 1.51)	1.20 (0.86, 1.67)	1.12 (0.78, 1.62)	0.45
<b>t/c-18:2</b>					
Cases (person-years)	77 (7,961)	79 (7,751)	66 (8,033)	65 (7,080)	—
Multivariate*	1.00 (ref)	1.12 (0.82, 1.55)	0.82 (0.59, 1.14)	0.96 (0.68, 1.35)	0.51
Multivariate + DNL FAs†	1.00 (ref)	1.17 (0.86, 1.62)	0.88 (0.63, 1.23)	1.07 (0.76, 1.51)	0.96
<b>t/t-18:2</b>					
Cases (person-years)	89 (8,698)	76 (7,797)	65 (7,182)	57 (7,149)	—
Multivariate*	1.00 (ref)	1.01 (0.74, 1.38)	0.93 (0.67, 1.29)	0.79 (0.55, 1.12)	0.18
Multivariate + DNL FAs†	1.00 (ref)	1.11 (0.81, 1.53)	1.08 (0.77, 1.52)	0.96 (0.66, 1.39)	0.81
<b>t-16:1n7</b>					
Cases (person-years)	96 (8,050)	78 (7,888)	66 (7,504)	47 (7,384)	—
Multivariate*	1.00 (ref)	0.87 (0.64, 1.17)	0.78 (0.57, 1.07)	0.63 (0.44, 0.91)	0.01
Multivariate + DNL FAs†	1.00 (ref)	0.95 (0.70, 1.29)	0.89 (0.64, 1.24)	0.73 (0.50, 1.06)	0.10

Data are HR (95% CI) unless otherwise indicated. Further adjustment for dietary factors (including consumption of coffee [caffeinated and decaffeinated], glycemic load, cereal fiber, polyunsaturated fat, saturated fat, magnesium, and circulating FAs [including plasma phospholipid n-6 fatty acids, 14:0, 15:0, 17:0, 16:1n7, 16:1n9, 18:1n7, and 18:1n9 FAs; t-16:1n7; eicosapentaenoic acid; docosahexaenoic acid;  $\alpha$ -linolenic acid; and other TFA subtypes]), use of statins, and metabolic factors (including levels of HDL cholesterol, triglycerides, blood insulin, and glucose) did not substantially alter the results (data not shown). DNL, de novo lipogenesis; FA, fatty acid; ref, reference. \*Adjusted for age, sex, race, education, enrollment site, smoking status, alcohol consumption, physical activity, BMI, waist circumference, prevalence of CVD, and hypertension at baseline. †Further adjusted for plasma phospholipid 16:0 and 18:0 FAs.

In animal models, high-TFA diets significantly reduce antilipolytic effects of insulin and insulin-stimulated glucose transport in adipocytes (4) and upregulate mRNA expression of resistin and downregulate expression of peroxisome proliferative-activated receptor  $\gamma$  and lipoprotein lipase (5), which could reduce insulin sensitivity (33,34). In vitro and in vivo, greater TFA exposure induces genetic expression of SREBPs and suppresses expression of triglyceride transfer protein, leading to stimulation of hepatic de novo lipogenesis and resulting in nonalcoholic steatohepatitis-like lesions, conditions closely linked to insulin resistance (8–10). Although no long-term interventions in humans have been performed, a 6-year feeding trial in nonhuman primates fed diets containing either *cis* fatty acids or TFAs (8% of energy) demonstrated postprandial hyperinsulinemia, elevated fructosamine, and trends toward higher glucose concentrations, reflecting impaired glucose disposal among TFA-fed

monkeys (35). Conversely, other shorter-term animal models found no significant changes in glucose or insulin levels with higher TFA exposure (6,7).

In baseline cross-sectional analyses, we found all circulating TFA levels to be strongly inversely associated with alcohol use and circulating 16:0 and 18:0 levels. The latter fatty acids are derived from both diet and de novo lipogenesis, and alcohol is a well-known driver of de novo lipogenesis (36). Of note, plasma phospholipid t-16:1n9 and t-18:1 were significantly associated with DM only after adjustment for circulating 16:0 and 18:0. Both the latter fatty acids and de novo lipogenesis are associated with a higher risk of DM (36–38). Because circulating 16:0 and 18:0 were also related to circulating TFA levels, this suggests important confounding by the former. The link between these circulating saturated fatty acids and TFA levels could be related to common dietary sources or perhaps an effect of de novo lipogenesis on relative circulating TFA concentrations. Because

absolute levels of circulating fatty acids vary widely between individuals (e.g., due to large differences in underlying lipid synthesis), fatty acid concentrations are typically assessed as relative proportions. A study of patients with alcoholism demonstrated that increased de novo lipogenesis raises relative proportions of 16:0 and 18:0, which could reduce relative concentrations of less abundant diet-derived fatty acids (39), such as TFA. The otherwise unexplained strong inverse associations between alcohol use, a driver of de novo lipogenesis, and each circulating TFA found in the current study as well as in the Nurses' Health Study and Zutphen Elderly Study are also consistent with this hypothesis (40,41). The current novel findings highlight the potential links among circulating TFA levels, saturated fatty acid levels, and incident DM, which could be at least partly explained by de novo lipogenesis. This study also warrants future investigations using absolute concentrations of circulating TFAs, which may be less influenced by levels of other

**Table 4—Prospective association of dietary TFAs with incident diabetes (n = 4,207)\***

	Quartile of dietary total and subclasses of TFA				P-trend value
	I	II	III	IV	
<b>Dietary total TFA (mean ± SD 3.7 ± 1.3 g/day)</b>					
Cases (total person-years)	85 (13,213)	104 (12,650)	98 (12,442)	120 (11,799)	—
Multivariate†	1.00 (ref)	1.11 (0.82, 1.50)	1.18 (0.87, 1.59)	1.38 (1.03, 1.86)	0.02
Multivariate + dietary factors‡	1.00 (ref)	1.11 (0.81, 1.52)	1.17 (0.85, 1.62)	1.38 (0.98, 1.93)	0.06
Multivariate + dietary score§	1.00 (ref)	1.08 (0.79, 1.48)	1.13 (0.81, 1.57)	1.31 (0.92, 1.86)	0.11
<b>Dietary 18:1 TFA (2.0 ± 0.7 g/day)</b>					
Cases (total person-years)	91 (13,309)	99 (12,727)	93 (12,248)	124 (11,821)	—
Multivariate†	1.00 (ref)	0.97 (0.72, 1.31)	1.06 (0.79, 1.42)	1.32 (0.99, 1.76)	0.04
Multivariate + dietary factors‡	1.00 (ref)	0.97 (0.71, 1.32)	1.05 (0.76, 1.45)	1.30 (0.93, 1.82)	0.09
Multivariate + dietary score§	1.00 (ref)	0.94 (0.86, 1.73)	1.01 (0.73, 1.39)	1.22 (0.86, 1.73)	0.18
<b>Dietary 18:2 TFA (0.5 ± 0.2 g/day)</b>					
Cases (total person-years)	92 (13,536)	93 (12,633)	98 (12,324)	124 (11,612)	—
Multivariate†	1.00 (ref)	1.05 (0.78, 1.41)	1.14 (0.85, 1.55)	1.41 (1.05, 1.89)	0.02
Multivariate + dietary factors‡	1.00 (ref)	1.05 (0.77, 1.44)	1.16 (0.84, 1.59)	1.44 (1.03, 2.01)	0.03
Multivariate + dietary score§	1.00 (ref)	1.02 (0.75, 1.40)	1.10 (0.80, 1.53)	1.34 (0.94, 1.90)	0.10
<b>Dietary 16:1 TFA (0.1 ± 0.03 g/day)</b>					
Cases (total person-years)	88 (12,699)	98 (12,869)	103 (12,451)	118 (12,087)	—
Multivariate†	1.00 (ref)	0.97 (0.72, 1.30)	1.04 (0.78, 1.41)	1.26 (0.94, 1.68)	0.06
Multivariate + dietary factors‡	1.00 (ref)	0.96 (0.71, 1.31)	1.03 (0.75, 1.42)	1.24 (0.88, 1.76)	0.18
Multivariate + dietary score§	1.00 (ref)	0.94 (0.70, 1.27)	0.99 (0.73, 1.35)	1.16 (0.84, 1.60)	0.21

Data are HR (95% CI) unless otherwise indicated. Further adjustment for consumption of cheese, yogurt, milk, and fish; magnesium; HDL cholesterol; and triglycerides did not substantially alter the results (data not shown). ref, reference. \*All participants with dietary TFA evaluated and without prevalent DM at baseline (baseline was set at the year when participants completed their initial dietary questionnaire). Dietary TFA consumption was cumulatively updated based on information from 1989–1990 and 1996 questionnaires; for those enrolled in 1992–1993, TFA consumption was collected from the 1996 questionnaire. A total of 407 new cases of DM were ascertained during 50,105 person-years of follow-up. †Adjusted for age, sex, race, education, enrollment site, smoking status, alcohol consumption, prevalence of physical activity, BMI, waist circumference, CVD, hypertension at baseline. ‡Further adjusted for consumption of coffee (caffeinated and decaffeinated), red meat, fiber, glycemic load, polyunsaturated fat, saturated fat, and total energy in the multivariate model. §Further adjusted for total energy, and the dietary score comprised consumption of whole grains, fish, fruits and vegetables, nuts and seeds, red and processed meat, sugar-sweetened beverages, and fried potatoes in the multivariate model.

endogenously synthesized fatty acids and better reflect TFA intakes than TFA levels measured as proportions of total fatty acids.

Although adjustment for 16:0 and 18:0 strengthened the findings for *t*-16:1n9 and *t*-18:1, it partly attenuated findings for *t*-16:1n7, a naturally occurring ruminant (e.g., dairy) fatty acid. Prior findings from both this cohort and a separate cohort of multiethnic U.S. adults demonstrated inverse associations between circulating *t*-16:1n7 and incident DM (21,22). After adjustment for circulating 16:0 and 18:0, a nonsignificant protective association was seen in the present cohort, and a significant inverse association was still evident in that separate cohort (M.C. de Oliveira, J.A. Nettleton, R.N. Lemaitre, et al., unpublished data). The present findings support the need for additional investigation of potential effects of circulating *t*-16:1n7 and its determinants on DM risk.

We found positive associations between total dietary TFA and DM risk after adjusting for major demographic, clinical, and lifestyle factors. However,

further adjusting for a composite dietary score attenuated the positive association. This is consistent with previous results among U.S. male health professionals where no significant association was found between estimated dietary TFA and DM (risk ratio [RR] 0.90, *P*-trend = 0.33) after adjustment for dietary factors (17), indicating confounding by other dietary habits. Conversely, another prospective cohort among U.S. nurses found a positive association (RR 1.31, *P*-trend = 0.02) (16), whereas in still another cohort among lowland females a protective association was observed (RR 0.83, *P*-trend = 0.004) (18). In exploratory analyses, positive trends were seen for both circulating and dietary *t*-18:1 with incident DM among participants with both higher insulin resistance and lower  $\beta$ -cell function, although these associations were nonsignificant and should be interpreted with caution. The current results suggest nonsignificant associations of dietary TFA with DM, highlighting the need for further investigation, especially using improved methods for estimating

dietary TFA and in well-designed interventional studies.

The low baseline correlation and, notably, the disparities of the findings between circulating and dietary TFAs can be attributed to various reasons. On the one hand, although circulating TFAs cannot be endogenously synthesized, suggesting that they partly reflect dietary intake, they are related to metabolic processes that are not well known. On the other hand, measurement errors and variations in TFA intake could result in a low correlation between circulating and dietary TFAs and an attenuation of the true associations of dietary TFAs.

This study has several strengths. Biomarker and dietary fatty acids, demographics, clinical factors, lifestyle, and metabolic risk factors were prospectively assessed in a well-established multicenter cohort with little loss to follow-up, establishing temporality, minimizing selection and recall bias, and increasing our ability to adjust for confounding. We investigated circulating TFA levels and estimated TFA consumption, providing complementary assessments of

exposure. Careful follow-up and multiple metrics for DM diagnosis minimized the potential for missed or misclassified outcomes.

Potential limitations should be highlighted. Circulating TFA levels were measured once at baseline, and within-person variation could have resulted in misclassification and attenuated true associations, especially for circulating *t*-18:2 isomers. TFA levels were expressed as a percentage of total fatty acids, which could be influenced by levels of other endogenous fatty acids independent of dietary TFA consumption. Although we used the best available and time-concordant dietary databases to estimate dietary TFA intake, measurement error in these estimates as well as changes in intake and product reformulations after 1995–1996 would result in the attenuation of the true associations. Because of the observational nature of the analysis, residual confounding by unknown or unmeasured factors cannot be excluded. Although we do not expect biological effects of TFA on DM to differ by age or race, the generalizability of the findings to other populations could be limited.

In conclusion, after adjustment for confounders, including de novo lipogenesis fatty acids, circulating *t*-16:1n9 and *t*-18:1 but not *t*-18:2 levels were positively associated with incident DM. These associations were not evident before adjustment for de novo lipogenesis fatty acids. Estimated dietary TFA was not significantly associated with higher risk after adjusting for other dietary habits, although the 95% CIs included the possibility of relevant harm. These findings highlight the need for further observational, interventional, and experimental studies to investigate potential effects TFA on DM.

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