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Trans-Palmitoleic Acid, Metabolic Risk Factors, and New-Onset Diabetes in U.S. Adults

A Cohort Study

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Background: Palmitoleic acid (*cis*-16:1n-7), which is produced by endogenous fat synthesis, has been linked to both beneficial and deleterious metabolic effects, potentially confounded by diverse determinants and tissue sources of endogenous production. *Trans*-palmitoleate (*trans*-16:1n-7) represents a distinctly exogenous source of 16:1n-7, unconfounded by endogenous synthesis or its determinants, that may be uniquely informative.

Objective: To investigate whether circulating *trans*-palmitoleate is independently related to lower metabolic risk and incident type 2 diabetes.

Design: Prospective cohort study from 1992 to 2006.

Setting: Four U.S. communities.

Patients: 3736 adults in the Cardiovascular Health Study.

Measurements: Anthropometric characteristics and levels of plasma phospholipid fatty acids, blood lipids, inflammatory markers, and glucose–insulin measured at baseline in 1992 and dietary habits measured 3 years earlier. Multivariate-adjusted models were used to investigate how demographic, clinical, and lifestyle factors independently related to plasma phospholipid *trans*-palmitoleate; how *trans*-palmitoleate related to major metabolic risk factors; and how *trans*-palmitoleate related to new-onset diabetes (304 incident cases). Findings were validated for metabolic risk factors in an independent cohort of 327 women.

Results: In multivariate analyses, whole-fat dairy consumption was most strongly associated with higher *trans*-palmitoleate levels. Higher *trans*-palmitoleate levels were associated with slightly lower adiposity and, independently, with higher high-density lipoprotein cholesterol levels (1.9% across quintiles; $P = 0.040$), lower triglyceride levels (−19.0%; $P < 0.001$), a lower total cholesterol–HDL cholesterol ratio (−4.7%; $P < 0.001$), lower C-reactive protein levels (−13.8%; $P = 0.05$), and lower insulin resistance (−16.7%, $P < 0.001$). *Trans*-palmitoleate was also associated with a substantially lower incidence of diabetes, with multivariate hazard ratios of 0.41 (95% CI, 0.27 to 0.64) and 0.38 (CI, 0.24 to 0.62) in quintiles 4 and 5 versus quintile 1 (P for trend < 0.001). Findings were independent of estimated dairy consumption or other fatty acid dairy biomarkers. Protective associations with metabolic risk factors were confirmed in the validation cohort.

Limitation: Results could be affected by measurement error or residual confounding.

Conclusion: Circulating *trans*-palmitoleate is associated with lower insulin resistance, presence of atherogenic dyslipidemia, and incident diabetes. Our findings may explain previously observed metabolic benefits of dairy consumption and support the need for detailed further experimental and clinical investigation.

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Fatty acids are powerful modulators of physiologic function, but the effects of many individual fatty acids are not well understood. Animal experiments suggest that circulating palmitoleic acid (*cis*-16:1n7), a product of endogenous fat synthesis, may directly regulate and protect against insulin resistance and metabolic dysregulation (1–5). Results from studies in humans have been mixed, with some observational studies suggesting protective and others deleterious associations between circulating palmitoleate and metabolic risk (6–20). Interpretation of these findings has been hampered by the diverse lifestyle determinants and tissue sources (such as liver or adipose tissue) of endogenous palmitoleate synthesis, which could confound or modify its metabolic effects (20).

Investigating an exogenous source would help clarify the role of palmitoleate in human metabolic risk. On the basis of our experimental work (1), we hypothesized that nonhepatic sources of palmitoleate may suppress hepatic fat synthesis and produce metabolic benefits. Differentiating adipose from hepatic sources of palmitoleate in large human cohorts is challenging, and palmitoleate from rare dietary sources (21) cannot be differentiated from endogenously synthesized palmitoleate as a circulating biomarker. In contrast, the *trans* isomer of palmitoleate (*trans*-16:1n-7) represents a distinctly exogenous source of 16:1n-7, unconfounded by endogenous synthesis or its determinants, that may be uniquely informative. Whereas trans fats from partially hydrogenated oils unfavorably affect cardiovascular risk (22), *trans*-palmitoleate is principally derived from naturally occurring dairy and other ruminant

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trans fats (23), consumption of which has not been associated with higher cardiovascular risk (22). In fact, several studies (24, 25) have demonstrated inverse associations between dairy consumption and risk for insulin resistance, the metabolic syndrome, or diabetes. To our knowledge, no previous studies have evaluated a potential role of *trans*-palmitoleate in metabolic risk.

We investigated the relationships between plasma phospholipid *trans*-palmitoleate and metabolic risk factors and incident type 2 diabetes among 3736 adults in the Cardiovascular Health Study (CHS). Validation for metabolic risk factors was performed in a second independent cohort of 327 women. We hypothesized that higher *trans*-palmitoleate levels would be associated with a better metabolic profile and a lower incidence of diabetes.

Context

The effects of fatty acids on metabolic outcomes are complex and may be influenced by such factors as whether they are endogenously produced or obtained exogenously from specific dietary sources. *Trans*-palmitoleate acid is a fatty acid obtained primarily from dairy consumption.

Contribution

This observational study found that higher levels of circulating *trans*-palmitoleate were associated with greater whole-fat dairy consumption, a more favorable metabolic profile, and a lower incidence of type 2 diabetes.

Caution

Trans-palmitoleate levels were measured only once. Residual confounding and measurement error in both exposures and covariates remain possible.

Implication

Further study is required to evaluate whether *trans*-palmitoleate has positive health effects.

—The Editors

METHODS

Design and Population

The CHS (26) is a prospective cohort study comprising 5201 ambulatory, noninstitutionalized adults 65 years or older (58% women and 42% men) who were randomly selected and enrolled from 1989 to 1990 from Medicare eligibility lists in 4 U.S. communities (Forsyth County, North Carolina; Sacramento County, California; Washington County, Maryland; and Allegheny County, Pennsylvania) and an additional 687 black adults who were similarly recruited and enrolled from these communities in 1992. Among all eligible adults contacted, 57% agreed to enroll. Trained personnel performed study clinic evaluations, using standardized methods, that included physical examination; diagnostic testing; laboratory evaluation; and questionnaires on health status, medical history, and cardiovascular and lifestyle risk factors. Participants were followed with annual clinic examinations and interim telephone contacts for 10 years, with telephone contacts every 6 months thereafter. The institutional review committee at each center approved the study, and all participants provided informed written consent.

Study Measures

Stored blood from the 1992 study clinic visit, which we considered the baseline for all present analyses, was available for fatty acid measurements. Blood was drawn after 12 hours of fasting, stored at −70 °C, and shipped on dry ice for centralized long-term storage at −80 °C. Among the 5565 living CHS participants in 1992, plasma phospholipid fatty acid levels were measured in 3736 participants (67%), comprising 3238 participants who were randomly selected from those with available blood samples and an additional 498 from a previous nested case–control study of incident heart disease within the CHS (27). Because these participants were not a random sample of all CHS participants, all analyses accounted for within-cohort sampling by using inverse probability of sampling weights. The 1992 visit and blood samples were used to assess all fatty acid levels, covariates, and metabolic outcomes except for dietary habits, which were assessed at enrollment 3 years earlier.

Fatty acid measurements were performed at the Fred Hutchinson Cancer Research Center, which provided quantitative measurement of 45 fatty acids as a percentage of total fatty acids. Plasma phospholipids represent a biomarker of longer term (4 to 8 weeks) circulating fatty acid levels, with similar responses to those of levels in erythrocyte membranes (28). No degradation, lipolysis, or oxidation has been observed after 10 years under the blood storage conditions in the CHS (29). Total lipids were extracted from plasma by using the methods of Folch and colleagues (30), and phospholipids were separated from neutral lipids by using 1-dimensional, thin-layer chromatography. Samples of fatty acid methyl ester were prepared by direct transesterification by using the methods of Lepage and Roy (31) and separated by using gas chromatography (5890 gas chromatograph flame ionization detector, Agilent Technologies, Palo Alto, California; SP-2560 fused-silica 100-m capillary column, Supelco, Belefonte, Pennsylvania; initial, 160 °C × 16 min; ramp, 3.0 °C/min to 240 °C; hold, 15 min). Identification, precision, and accuracy were continuously evaluated by using model mixtures of known fatty acid methyl esters and established in-house control samples, with identification confirmed by the U.S. Department of Agriculture or, for trans fats, by silver ion thin-layer chromatography. Laboratory coefficients of variation were 3.0% for *trans*-palmitoleate and less than 3% for most fatty acids. We assessed long-term reproducibility of *trans*-palmitoleate levels in a subset of 100 participants that would capture laboratory error, biologic variability, and dietary changes over time. Correlations with baseline levels

were 0.64 at 6 years and 0.40 at 13 years, which are similar to within-person correlations over time for other common risk factors, such as blood pressure (32).

Anthropometric measures, including weight, height, and waist circumference, were collected by using standard procedures and equipment. Fasting blood lipid levels were measured according to Centers for Disease Control and Prevention methods, and low-density-lipoprotein (LDL) cholesterol levels were calculated by using the Friedewald equation; participants with hypertriglyceridemia were excluded. Fasting glucose and insulin levels (as measured by an Ektacham 700 Analyzer, Eastman Kodak, Rochester, New York) were used to derive the homeostasis assessment model of insulin resistance (glucose [mg/dL] × insulin [mU/L]) ÷ 405). Fibrinogen was measured by using standard methods, and C-reactive protein (CRP) was measured by using a validated, in-house, high-sensitivity enzyme-linked immunosorbent assay. Standardized questionnaires assessed usual frequency and types of alcoholic beverages consumed (wine, beer, or liquor). Leisure activity was assessed by using a modified Minnesota Leisure-Time Activities questionnaire that evaluated the frequency and duration of 15 activities. Diet was assessed by using a picture-sort food frequency questionnaire validated against 6 detailed 24-hour diet recalls spaced approximately 1 month apart (33).

At annual study clinic visits, participants reported all prescription medications taken in the previous 2 weeks; after 10 years of follow-up, similar information was collected annually by telephone. Detailed medication data, including drug names, doses, and frequencies coded according to prescription Medispan files, were obtained and recorded by using computerized inventories. Prevalent and incident diabetes were defined by medication use and the results of CHS blood testing. Prevalent diabetes was defined as use of insulin or hypoglycemic medication, fasting glucose levels of 7.0 mmol/L (126 mg/dL) or greater, or (among the 1.7% of participants who fasted <8 hours) nonfasting glucose levels of 11.1 mmol/L (200 mg/dL) or greater. Incident diabetes was defined by new use of insulin or hypoglycemic medication (assessed annually), fasting glucose levels of 7.0 mmol/L (126 mg/dL) or greater (assessed in 1996), or 2-hour postchallenge glucose levels of 11.1 mmol/L (200 mg/dL) or greater (assessed in 1996). Medication information was complete for 96.4% of person-time; vital status follow-up was 100% complete.

Validation Cohort

For independent validation, we evaluated the association of *trans*-palmitoleate, measured in erythrocyte membranes and plasma (34), with metabolic risk factors in a cohort of 327 generally healthy women (mean age, 60.4 years [SD, 6.1]) in the Nurses Health Study. Relationships were evaluated for levels of high-density lipoprotein (HDL) cholesterol, interleukin-6, and CRP; ratio of total cholesterol to HDL cholesterol; and hemoglobin A_{1c}. Because many samples were nonfasting, triglyceride and LDL cholesterol levels were not evaluated; in addition, the number of patients was too small to evaluate incident diabetes. Multivariate models were adjusted for age; smoking; physical activity; alcohol intake; family history; hypertension; hormone use; fasting status; and dietary carbohydrates, protein, polyunsaturated fats, saturated fat, fiber, and total energy. We also evaluated correlations of *trans*-palmitoleate levels in plasma versus erythrocytes in the Nurses Health Study ($r = 0.74$) and in phospholipid versus triglyceride levels in the CHS (104 participants; $r = 0.54$).

Statistical Analysis

We evaluated *trans*-palmitoleate levels in sex-specific quintiles as indicator variables and continuously by SD difference (0.05–percentage point change). Independent demographic and lifestyle factors associated with *trans*-palmitoleate level were assessed by using multivariate-adjusted linear regression, with *trans*-palmitoleate level as the dependent variable. Multivariate-adjusted relationships of *trans*-palmitoleate level with metabolic risk factors (transformed for normality as appropriate) were evaluated by using linear regression, with *trans*-palmitoleate level as the independent variable. Quintiles were evaluated as ordinal variables to assess trend and effect modification by using the Wald test for a multiplicative interaction term. We used a Cox proportional hazards model to estimate the hazard ratio (HR) of incident diabetes, with time at risk until first diagnosis, last follow-up visit with medication information, or administrative censoring in 2006 as the latest date of adjudicated medication data. The proportional hazards assumption was not rejected on the basis of Schoenfeld residuals. To minimize potential confounding, covariates were selected on the basis of biological interest, being well-established risk factors for metabolic risk, or associations with exposures or outcomes in the cohort. Missing covariates (≤1.9% for most factors and 7% to 10% for dietary factors) were imputed by best-subset regression by using baseline age, sex, race, education, coronary heart disease (CHD), stroke, diabetes, smoking status, alcohol use, physical activity, body mass index, and other relevant dietary variables for nutritional factors; results from using multiple imputation (35) or excluding missing values were similar. Analyses were performed by using Stata, version 10.1 (StataCorp, College Station, Texas), with a 2-tailed α of 0.05.

Role of the Funding Source

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Table 1. **Baseline Characteristics, by Plasma Phospholipid *Trans*-Palmitoleic Acid Level***

Characteristic	Sex-Specific Quintiles of *Trans*-Palmitoleic Acid Level				
	1	2	3	4	5
Patients, *n*	767	738	744	748	739
Mean total fatty acids (SD), %	0.12 (0.02)	0.16 (0.01)	0.18 (0.01)	0.21 (0.01)	0.26 (0.03)
Mean age (SD), *y*	75 (5)	75 (5)	75 (5)	76 (5)	76 (5)
Men, %	43	47	45	46	46
White, %	81	84	86	90	91
Education beyond high school, %	42	43	46	47	46
Current smokers, %	9	6	8	7	10
Coronary heart disease, %	26	27	26	23	19
Diabetes mellitus, %	17	18	19	17	13
Moderate or greater exercise intensity, %	44	42	45	46	44
Mean leisure activity (SD), *kcal/wk*	1084 (1422)	1056 (1433)	1170 (1475)	1063 (1471)	943 (1269)
Mean alcohol consumption (SD), *drinks/wk*	3.7 (10.3)	2.2 (4.9)	1.8 (4.6)	1.6 (4.1)	1.3 (3.9)
Mean total fat (SD), *% energy*	30.7 (6)	31.6 (6)	32.0 (6)	32.8 (5)	32.9 (6)
Mean carbohydrates (SD), *% energy*	53.8 (8)	52.8 (8)	52.4 (7)	51.6 (7)	51.5 (7)
Mean intake of whole-fat dairy (SD), *servings/wk*†	5.7 (4.3)	6.5 (4.8)	6.7 (4.5)	7.9 (4.9)	8.7 (5.4)
Mean intake of low-fat dairy (SD), *servings/wk*†	4.1 (3.7)	4.3 (3.9)	3.8 (3.4)	3.8 (3.4)	3.6 (3.7)
Mean intake of red meat (SD), *servings/wk*	2.4 (2.2)	2.5 (2.1)	2.8 (2.2)	3.1 (2.5)	3.2 (2.5)

* All characteristics were assessed at the 1992 baseline study visit except for diet, which was assessed 3 y earlier.
† Whole-fat dairy foods include whole milk, 2% milk, cheese, butter, and ice cream. Low-fat dairy foods include 1% milk, skim milk, cottage cheese, and yogurt.

RESULTS

Trans-palmitoleate represented less than 1% of total fatty acids (mean, 0.18% [SD, 0.05%]; range, 0.02% to 0.55%). *Trans*-palmitoleate levels correlated strongly with levels of fatty acid biomarkers of dairy fat consumption (36–38), such as 15:0 ($r = 0.64$) and 17:0 ($r = 0.66$), but correlated weakly with levels of trans fats that are frequently derived from partially hydrogenated oils (23), such as *trans*-16:1n-9 ($r = 0.11$), *trans*-18:1n-7 ($r = 0.25$), total *trans*-18:1 ($r = 0.15$), or total *trans*-18:2 ($r = 0.07$). This is consistent with dairy foods—rather than industrially produced trans fats—being a major source of *trans*-palmitoleate. In bivariate (unadjusted) analyses (**Table 1**), circulating *trans*-palmitoleate levels were associated with slightly older age, white race, and modestly less prevalent CHD. They were also associated with less alcohol use, modestly higher total fat intake, modestly lower carbohydrate and low-fat dairy consumption, and greater consumption of whole-fat dairy foods and red meat.

We clarified and quantified the factors that were independently associated with circulating *trans*-palmitoleate levels in this large community-based cohort (**Table 2**). In multivariate analyses, older age, nonwhite race, and greater education were associated with higher levels of circulating *trans*-palmitoleate and female sex and prevalent CHD were associated with lower levels. Greater body mass index and alcohol use were also associated with slightly lower levels. Whole-fat dairy consumption had the strongest relationship, with 0.69-SD higher *trans*-palmitoleate levels among participants who consumed 15 or more servings per week (≥2 servings per day) versus 2 or fewer servings per week. An evaluation of different dairy foods suggested that this relationship was directly related to dairy fat content, with the strongest association for whole milk (0.49 higher SD of *trans*-palmitoleate per serving per day; $P < 0.001$), then butter (0.31; $P < 0.001$), 2% milk (0.21; $P < 0.001$), cheese (0.20; $P = 0.041$), and ice cream (0.18; $P = 0.093$).

In multivariate-adjusted analyses, *trans*-palmitoleate was significantly associated with several metabolic risk factors (**Table 3**). Higher *trans*-palmitoleate levels were associated with slightly lower body mass index (−1.8% across quintiles; $P = 0.058$) and waist circumference (−1.8%; $P = 0.009$). In multivariate models that were further adjusted for adiposity, *trans*-palmitoleate was associated with slightly higher HDL cholesterol levels (1.9%; $P = 0.043$); substantially lower triglyceride levels (−19.0%; $P < 0.001$); a lower total cholesterol–HDL cholesterol ratio (−4.7%; $P < 0.001$); and lower levels of CRP (−13.8%; $P = 0.050$), a marker of systemic inflammation related to risk for the metabolic syndrome and diabetes (39, 40). *Trans*-palmitoleate was also associated with fibrinogen, an acute-phase reactant elevated by insulin resistance (41), but no dose–response effect was observed; lower levels were seen only in the lowest quintile. *Trans*-palmitoleate was associated with lower fasting insulin levels (−13.3%; $P < 0.001$) and insulin resistance (homeostasis model assessment, −16.7%; $P < 0.001$). *Trans*-palmitoleate was not associated with LDL cholesterol or fasting glucose levels or with blood pressure. All relationships seemed similar regardless of the sex of the participants (data not shown).

Among the 2985 participants who did not have prevalent diabetes at baseline, 304 new cases occurred during 27 866 person-years. In age- and sex-adjusted analyses, circulating *trans*-palmitoleate levels were associated with lower risk for new-onset diabetes, including a 2-fold lower risk in quintile 4 (HR, 0.44 [95% CI, 0.30 to 0.66]) and a 3-fold lower risk in quintile 5 (HR, 0.36 [CI, 0.23 to

Table 2. **Multivariate-Adjusted Relationships of Demographic, Clinical, and Lifestyle Factors With Plasma Phospholipid *Trans*-Palmitoleic Acid**

Factor	SD Difference in *Trans*-Palmitoleic Acid Level (95% CI)*	*P* Value
Age, each 5 y	0.08 (0.04 to 0.12)	<0.001
Sex		
Male	Reference	
Female	−0.25 (−0.34 to −0.16)	<0.001
Race		
White	Reference	
Nonwhite	0.33 (0.19 to 0.48)	<0.001
Education		
Less than high school	Reference	
High school graduate	0.07 (−0.04 to 0.18)	0.22
Some college	0.14 (0.02 to 0.27)	0.019
College graduate	0.16 (0.03 to 0.29)	0.017
Prevalent diabetes		
No	Reference	
Yes	−0.03 (−0.14 to 0.09)	0.63
Prevalent coronary heart disease		
No	Reference	
Yes	−0.14 (−0.23 to −0.04)	0.007
Smoking status		
Never	Reference	
Former	−0.04 (−0.13 to 0.05)	0.41
Current	0.09 (−0.06 to 0.24)	0.26
Body mass index, each kg/m^2	−0.01 (−0.02 to 0.00)	0.004
Leisure activity, each 500 kcal/wk	−0.01 (−0.02 to 0.01)	0.28
Alcohol intake, each drink/wk	−0.03 (−0.04 to −0.02)	<0.001
Carbohydrate intake, each higher 5% energy-replacing fat	−0.05 (−0.08 to −0.01)	0.014
Protein intake, each higher 5% energy-replacing fat	−0.08 (−0.16 to 0.00)	0.045
Whole-fat dairy intake		
0–2 servings/wk	Reference	
3–5 servings/wk	0.15 (0.01 to 0.28)	0.036
6–8 servings/wk	0.21 (0.08 to 0.34)	0.001
9–11 servings/wk	0.41 (0.27 to 0.55)	<0.001
12–14 servings/wk	0.40 (0.24 to 0.56)	<0.001
≥15 servings/wk	0.69 (0.49 to 0.89)	<0.001
Low-fat dairy intake, each serving/d	−0.01 (−0.03 to 0.00)	0.034
Red meat intake, each serving/d	0.14 (0.00 to 0.27)	0.058

* Values are multivariate-adjusted associations of each factor with *trans*-palmitoleic acid levels, evaluated per 1 SD (0.05–percentage point difference in total fatty acid level), adjusted for each variable simultaneously. A value of 1.00 would represent a 1-SD difference in *trans*-palmitoleate levels associated with the factor. All characteristics were assessed at the 1992 baseline study visit except for diet, which was assessed 3 y earlier.

0.57]) versus quintile 1 (P for trend < 0.001) (**Table 4**). Further adjustment for demographic, clinical, and lifestyle factors, including dairy and red meat consumption, did not appreciably alter results (**Table 4**). The lower risk associated with *trans*-palmitoleate levels seemed similar for both men (extreme-quintile HR, 0.29 [CI, 0.11 to 0.74]) and women (extreme-quintile HR, 0.41 [CI, 0.24 to 0.71]) (P for interaction = 0.87). When evaluated continuously per SD (0.05–percentage point difference in total fatty acid level), each higher SD of *trans*-palmitoleate was associated with a 28% lower risk for diabetes (multivariate-adjusted HR, 0.72 [CI, 0.61 to 0.86]; $P < 0.001$). To explore potential reverse causation (alteration of *trans*-palmitoleate levels due to the presence of subclinical disease), we performed sensitivity analyses that excluded 1106 participants with fasting glucose levels of 5.56 mmol/L (100 mg/dL) or higher at baseline. *Trans*-palmitoleate levels remained inversely associated with incident diabetes (extreme-quintile HR, 0.31 [CI, 0.13 to 0.71]).

On the basis of both biological considerations and our current findings (**Table 2**), consumption of carbohydrates, protein, red meat, and dairy foods could each confound relations between *trans*-palmitoleate levels and incident diabetes, even in fully multivariate-adjusted analyses. We first evaluated whether these factors themselves were independently associated with incident diabetes. After adjustment for the covariates listed in **Table 4** but excluding adjustment for *trans*-palmitoleate levels, consumption of carbohydrates ($P = 0.81$), protein ($P = 0.88$), red meat ($P = 0.69$), or low-fat dairy foods ($P = 0.98$) was not associated with incident diabetes. Conversely, greater whole-fat dairy consumption was associated with lower risk for diabetes across 6 categories (P for trend = 0.024). However, when we further adjusted for *trans*-palmitoleate levels, this association was attenuated and no longer significant (P for trend = 0.162). In contrast, the relationship of *trans*-palmitoleate levels with diabetes risk was robust to adjustment for dairy consumption (**Table 4**).

Our findings suggested that *trans*-palmitoleate, rather than consumption of specific foods, was the principal factor related to diabetes risk. However, *trans*-palmitoleate levels were measured objectively, whereas food intakes were estimated by questionnaire. We evaluated whether other fatty acid dairy biomarkers related to diabetes risk (15:0, 17:0), as well as whether associations of *trans*-palmitoleate levels with diabetes risk persisted after adjustment for these biomarkers; we also similarly evaluated *trans*-18:1n-7 (vaccenic acid), which is present in both dairy fats (42) and other food sources (23). After multivariate adjustment, 15:0 (HR, 0.99 [CI, 0.89 to 1.10]), 17:0 (HR, 0.98 [CI, 0.85 to 1.13]), and vaccenic acid (HR, 0.97 [CI, 0.93 to 1.01]) all showed no association with diabetes risk (HRs were evaluated per 0.05–percentage point difference in total fatty acid level, which corresponded to 1.0, 0.7, and 0.3 SD differences, respectively). However, after simultaneous adjustment for levels of 15:0, 17:0, and vaccenic acid, *trans*-palmitoleate levels remained inversely associated with new-onset diabetes (extreme-quintile HR, 0.43 [CI, 0.25 to 0.75]; P for trend < 0.001).

In our separate validation cohort, higher erythrocyte *trans*-palmitoleate levels were associated with lower levels of interleukin-6 (multivariate-adjusted levels across quartiles, 2.3 vs. 1.8 ng/L; P for trend $= 0.020$) and CRP (31.4 vs. 20.0 nmol/L; P for trend $= 0.020$) and trends toward lower total cholesterol–HDL cholesterol ratios (4.21 vs. 3.87; P for trend $= 0.099$) and hemoglobin A_{1c} (5.92% vs. 5.66%; P for trend $= 0.056$). In similar analyses, higher plasma *trans*-palmitoleate levels were associated with higher levels of HDL cholesterol (1.46 vs. 1.60 mmol/L [56.5 vs. 61.7 mg/dL]; P for trend $= 0.033$) and lower total cholesterol–HDL cholesterol ratios (4.28 vs. 3.63; P for trend < 0.001) and hemoglobin A_{1c} (5.88% vs. 5.64%; P for trend $= 0.028$).

Table 3. **Multivariate-Adjusted Relationships of Plasma Phospholipid *Trans*-Palmitoleic Acid With Metabolic Risk Factors***

Factor	Quintiles of *Trans*-Palmitoleic Acid Level					*P* Value for Trend
	1	2	3	4	5	
Median total fatty acid level, %	0.13	0.16	0.18	0.21	0.25	
Adiposity						
Body mass index, *kg/m²*	26.7	27.0	26.8	26.9	26.2	0.058
Waist circumference, *cm*	97.7	98.4	97.2	97.4	96.0†	0.009
Blood lipids						
LDL cholesterol level						0.63
mmol/L	3.26	3.32	3.29	3.26	3.34	
mg/dL	126	128	127	126	129	
HDL cholesterol level						0.043
mmol/L	1.37	1.35	1.35	1.39	1.40	
mg/dL	53.0	52.0	52.1	53.7	54.0	
Triglyceride level						<0.001
mmol/L	1.66	1.51	1.45	1.36	1.34	
mg/dL	147	134‡	128§	120§	119§	
Total cholesterol–HDL cholesterol ratio	4.3	4.3	4.2	4.1‡	4.1‡	<0.001
Inflammation						
C-reactive protein level, *nmol/L*	27.6	26.7	24.8	25.7	23.8	0.050
Fibrinogen level, *μmol/L*	9.3	9.6‡	9.7‡	9.8†	9.6‡	0.006
Glucose–insulin homeostasis						
Fasting glucose level						0.103
mmol/L	5.7	5.8	5.7	5.7	5.7	
mg/dL	104	105	103	103	103	
Fasting insulin level, *pmol/L*	78.5	76.4	74.3	70.8‡	68.1§	<0.001
Insulin resistance, *units*‖	3.0	2.9	2.8†	2.7‡	2.5§	<0.001
Blood pressure, *mm Hg*						
Systolic	137	136	136	136	136	0.48
Diastolic	71	71	70	71	71	0.98

HDL = high-density lipoprotein; LDL = low-density lipoprotein.
* Values are adjusted means, transformed to approximate normality for analyses and retransformed as necessary, adjusted for age (y); sex; race (white or nonwhite); education (less than high school, high school, some college, or college graduate); enrollment site (4 sites); smoking status (never, former, or current); diabetes (yes or no); coronary heart disease (yes or no); physical activity (kcal/wk); alcohol use (6 categories); and consumption of carbohydrates (% energy), protein (% energy), red meat (servings/wk), whole-fat dairy foods (6 categories), low-fat dairy foods (5 categories), and total energy (kcal/d). Results for measures of blood lipids, inflammation, glucose–insulin homeostasis, and blood pressure were also adjusted for body mass index (kg/m^2) and waist circumference (cm).
† $P < 0.050$ compared with quintile 1.
‡ $P < 0.010$ compared with quintile 1.
§ $P < 0.001$ compared with quintile 1.
‖ As measured by the homeostasis model assessment.

Table 4. **Incidence of Diabetes Mellitus Between 1992 and 2006, by Plasma Phospholipid *Trans*-Palmitoleic Acid Level***

Variable	Quintiles of *Trans*-Palmitoleic Acid Level					*P* Value for Trend
	1	2	3	4	5	
Person-years of follow-up	5595	5435	5556	5811	5469	
Incident cases, *n*	93	68	59	46	38	
Hazard ratio (95% CI)						
Age- and sex-adjusted	1.0 (reference)	0.84 (0.57–1.22)	0.88 (0.59–1.31)	0.44 (0.30–0.66)	0.36 (0.23–0.57)	<0.001
Multivariate-adjusted	1.0 (reference)	0.79 (0.54–1.15)	0.89 (0.58–1.33)	0.41 (0.27–0.64)	0.38 (0.24–0.62)	<0.001

* Incident diabetes was defined by new use of insulin or oral hypoglycemic medication, fasting glucose level ≥7.0 mmol/L (≥126 mg/dL), or 2-h postchallenge glucose level ≥11.1 mmol/L (≥200 mg/dL), excluding participants with prevalent diabetes at baseline. Multivariate model adjusted for age (y); sex; race (white or nonwhite); education (less than high school, high school, some college, or college graduate); enrollment site (4 sites); smoking status (never, former, or current); body mass index (kg/m^2); waist circumference (cm); coronary heart disease (yes or no); physical activity (kcal/wk); alcohol use (6 categories); and consumption of carbohydrates (% energy), protein (% energy), red meat (servings/wk), whole-fat dairy foods (6 categories), low-fat dairy foods (5 categories), and total energy (kcal/d).

DISCUSSION

In this large prospective cohort, phospholipid *trans*-palmitoleate levels were independently associated with lower metabolic risk. *Trans*-palmitoleate was associated with slightly lower adiposity and, independently, with higher HDL cholesterol levels; lower triglyceride levels, total cholesterol–HDL cholesterol ratio, and insulin resistance; and substantially lower onset of diabetes, with a nearly 3-fold lower risk across quintiles. The magnitude and robustness of these relationships were both substantial. The observed relationships were independent of adjustment for a number of demographic, clinical, lifestyle, and dietary factors, including other dairy fat biomarkers. Neither dairy foods nor other phospholipid biomarkers of dairy consumption were independently associated with diabetes risk, which supports a specific relationship between *trans*-palmitoleate levels, rather than dairy consumption in general, and diabetes. Inverse associations with diabetes-related metabolic risk factors were confirmed in a separate validation cohort. To our knowledge, our findings represent the first report of how *trans*-palmitoleate relates to metabolic risk markers and incident diabetes.

On the basis of our English-language MEDLINE search through June 2010, experimental effects of *trans*-palmitoleate on metabolic risk have not been reported, which precludes a direct evaluation of potential mechanisms of the presently observed metabolic benefits. In our study, *trans*-palmitoleate was unassociated with LDL cholesterol levels or blood pressure, which suggests specificity for atherogenic dyslipidemia and insulin resistance pathways rather than all metabolic pathways or better general health. Our findings for circulating *trans*-palmitoleate, a largely unstudied fatty acid produced by ruminant stomach bacteria and consumed in dairy and meats, parallel the metabolic protection of circulating *cis*-palmitoleate that we observed when we experimentally upregulated adipose tissue production of *cis*-palmitoleate (1). In that animal model (1), adipose-produced *cis*-palmitoleate directly improved hepatic and skeletal muscle insulin resistance and related metabolic abnormalities while also suppressing hepatic fat synthesis. The latter findings suggested that circulating palmitoleate derived from nonhepatic sources might provide counterregulatory feedback against hepatic fat synthesis. Considerable experimental evidence (43–45) suggests that increased hepatic fat synthesis contributes to nonalcoholic steatohepatitis and associated insulin resistance. We speculate that *trans*-palmitoleate, as an exogenous, nonhepatic source of palmitoleate, may partly suppress hepatic fat synthesis or have other beneficial physiologic effects (such as augmenting skeletal muscle glucose uptake) by mimicking or competing with the pathways of effect of either *cis*-palmitoleate (which has a similar molecular structure but different bond configuration) or 16:0 (which has a different bond structure but similar stereochemical shape). For example, *trans*-palmitoleate could mimic a putative counterregulatory role of adipose-produced *cis*-palmitoleate that has been largely lost in typical modern diets, in which high carbohydrates and excess energy are the key stimulators of hepatic fat synthesis (28, 46–50).

Limited evidence suggests that ruminant trans fats can regulate fat synthesis. In bovine models, dietary *trans*-10/*cis*-12 conjugated linoleic acid inhibited mammary gland fat synthesis by decreasing expression of lipogenic genes (51), with compensatory upregulation of adipose tissue genes involved in fat synthesis (52). Although no previous studies have reported on *trans*-palmitoleate and metabolic risk, a small study of 191 patients with established CHD (53) showed a nonsignificant inverse trend between platelet *trans*-palmitoleate levels and coronary atherosclerosis ($P = 0.12$), whereas other trans fats were associated with greater atherosclerosis. Given our present observations, the potential metabolic effects of *trans*-palmitoleate present a promising area for future investigation.

Our results may offer insights into some previous observations. First, consumption of ruminant trans fat has not been associated with higher cardiovascular risk; 3 cohorts have observed nonsignificant trends toward inverse associations (22). These findings remain unexplained, because major ruminant trans fats (predominantly *trans*-18:1 isomers) seem to adversely affect blood cholesterol levels similar to equivalent doses of industrial trans fats (54). Our

findings suggest that *trans*-palmitoleate, a fatty acid that is nearly unique to ruminant foods (23), could at least partly offset the adverse effects of other trans fats in ruminant foods. In addition, several large cohorts (24, 25, 55–63) have recently reported inverse associations between dairy consumption and risk for obesity, metabolic risk factors, or type 2 diabetes without consistent differences for various types of dairy foods (57–61). Vaccenic acid and calcium were proposed mediators of such benefits, but vaccenic acid and its metabolites (such as conjugated linoleic acid) produced disappointingly adverse effects on blood lipid levels and insulin resistance (64–67), and clinical studies that evaluated dairy calcium (68, 69) found little or no metabolic benefits. Our findings support potential metabolic benefits of dairy consumption and suggest that *trans*-palmitoleate may mediate these effects. They also suggest that efforts to promote exclusive consumption of low-fat and nonfat dairy products, which would lower population exposure to *trans*-palmitoleate, may be premature until the mediators of the health effects of dairy consumption are better established.

Our analysis has several strengths. Information on fatty acid levels, metabolic risk factors, covariates, and diabetes incidence were prospectively collected in a well-established multicenter study with close follow-up. Biomarker fatty acids provided objective measures of exposure. Large numbers of participants increased statistical power. Participants were randomly selected and enrolled from Medicare eligibility lists in several U.S. communities, which provided a community-based sample and increased generalizability. Many demographic, lifestyle, and dietary covariates were available for multivariable adjustment, which minimized residual confounding.

Our analysis also has limitations. The associations with metabolic risk factors were cross-sectional, which limits the assessment of temporality. However, prospective analyses of diabetes incidence were strongly supportive. Measurement error and biological variability were present in exposures and covariates, which could have biased results in unpredictable directions. Although we adjusted for major metabolic risk factors, residual confounding by unmeasured or imperfectly measured factors may be present. Conversely, the magnitudes of our multivariate-adjusted findings, including the nearly 3-fold lower incidence of diabetes, makes it improbable that residual confounding fully accounts for these relationships. In addition, the protective associations were independent of estimated dairy consumption and other dairy fatty acid biomarkers. Blood glucose level was not measured annually, and diabetes incidence might be underestimated. Several relationships and metabolic risk factors were evaluated as outcomes, and *P* values should therefore be considered as a guide.

Our results demonstrate an inverse relationship between levels of *trans*-palmitoleate and metabolic risk factors and diabetes incidence. The small differences in *trans*-palmitoleate levels raise questions about whether this is the active compound or a marker for some other, unknown protective constituent of dairy or other ruminant foods. However, they also suggest that if it is causal, this fatty acid is a potential candidate for enrichment of dairy foods or supplementation. Our findings support a role of this fatty acid in previously observed metabolic benefits of dairy consumption, with pathways potentially related to insulin resistance, atherogenic dyslipidemia, and regulation of hepatic fat synthesis. These results support the need for additional detailed experimental and clinical investigation, including animal experiments and metabolic feeding studies, to assess the potential health effects of *trans*-palmitoleate.

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