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Trans-Palmitoleic Acid, Metabolic Risk Factors, and New-Onset Diabetes in US Adults

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Abstract

Background—Palmitoleic acid (*cis*-16:1n-7), 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.

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Conflict of Interest Disclosures: Harvard University has filed a provisional patent application, that will be assigned to Harvard University, that lists Drs. Mozaffarian, Cao, and Hotamisligil as inventors to the US Patent and Trademark Office for use of trans-16:1n-7 to prevent and treat insulin resistance, type 2 diabetes, and related conditions.

Author Contributions:
Dr. Mozaffarian: Conception and design, obtained funding, data collection, statistical analysis, data interpretation, manuscript drafting, manuscript critical revision, and approval of final submitted manuscript.
Drs. Cao and Hotamisligil: Conception and design, data interpretation, critical revision of the manuscript, and approval of final submitted manuscript.
Dr. King: Obtained funding, data collection, data interpretation, critical revision of the manuscript, and approval of final submitted manuscript.
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Availability to Readers:
Study Protocol: Available to interested readers by contacting Dr. Mozaffarian at dmozaffa@hsph.harvard.edu
Statistical Code: Available to interested readers by contacting Dr. Mozaffarian at dmozaffa@hsph.harvard.edu
Data: Available to interested readers through established Cardiovascular Health Study procedures for obtaining and analyzing data; see www.chs-nhlbi.org/CHS_DistribPolicy.htm

Objective—We investigated whether circulating trans-palmitoleate was independently related to lower metabolic risk and incident type2 diabetes.

Design—Prospective cohort study (1992–2006).

Setting—Four US communities.

Patients—3,736 adults in the Cardiovascular Health Study.

Measurements—Plasma phospholipid fatty acids, anthropometry, blood lipids, inflammatory markers, and glucose-insulin levels were measured at baseline in 1992; and diet, 3 years earlier. In multivariable-adjusted models, we investigated how demographic, clinical, and lifestyle factors independently related to trans-palmitoleate; how trans-palmitoleate related to major metabolic risk factors; and how trans-palmitoleate related to new-onset diabetes (304 incident cases). We validated findings for metabolic risk factors in an independent cohort of 327 women.

Results—In multivariable-analyses, whole-fat dairy consumption was most strongly associated with higher trans-palmitoleate. Higher trans-palmitoleate was associated with slightly lower adiposity and, independently, higher high-density-lipoprotein(HDL)-cholesterol (across quintiles: +1.9%, P=0.04), lower triglycerides (−19.0%, P<0.001), lower total:HDL-cholesterol (−4.7%, P<0.001), lower C-reactive protein (−13.8%, P=0.05), and lower insulin resistance (−16.7%, P<0.001). Trans-palmitoleate was associated with substantially lower incidence of diabetes, with multivariable-hazard-ratios=0.41 (95%CI=0.27–0.64) and 0.38 (95%CI=0.24–0.62) in quintile-4 and quintile-5, versus quintile-1 (P-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.

Limitations—Measurement error; residual confounding.

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

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INTRODUCTION

Fatty acids are powerful modulators of physiologic function, but 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) 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 as well as tissue sources (e.g., liver, adipose tissue) of endogenous palmitoleate synthesis, that could confound or modify its metabolic effects.(20)

Investigation of an exogenous source would help elucidate palmitoleate's role in human metabolic risk. Based on our experimental work,(1) we hypothesized that non-hepatic sources of palmitoleate may suppress hepatic fat synthesis and produce metabolic benefits. Differentiating adipose vs. hepatic sources in large human cohorts is challenging; further, 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/ruminant *trans*-fats,(23) consumption of which has not been associated with higher cardiovascular risk.(22) Indeed, several cohorts have seen inverse associations between dairy consumption and risk of insulin resistance, metabolic syndrome, and diabetes.(24,25) No prior studies have evaluated a potential role of trans-palmitoleate in metabolic risk.

We investigated relationships between plasma phospholipid trans-palmitoleate and metabolic risk factors and incident type2 diabetes among 3,736 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 lower incidence of diabetes.

METHODS

Design and Population

CHS is a prospective cohort study among older adults (58% women, 42% men).(26) Briefly, 5,201 ambulatory, non-institutionalized adults≥age 65 were randomly selected and enrolled in 1989–90 from Medicare eligibility lists in 4 U.S. communities (Forsyth County, North Carolina; Sacramento County, California; Washington County, Maryland; Allegheny County, Pennsylvania); an additional 687 black participants were similarly recruited and enrolled from these communities in 1992. Among all eligible adults contacted, 57% agreed to enroll. Study-clinic evaluations were performed by trained personnel using standardized methods and included physical examination, diagnostic testing, laboratory evaluation, and questionnaires on health status, medical history, and cardiovascular and lifestyle risk factors. Participants were followed by means of annual study-clinic examinations with interim phone contacts for 10 years, with telephone contacts every 6 months thereafter. Each center's institutional review committee approved the study; all participants provided informed written consent.

Study Measures

Stored blood was available for fatty acid measurements from the 1992 study-clinic visit, considered baseline for all present analyses. Blood was drawn after 12-hours fasting, stored (−70°C), and shipped on dry ice for centralized long-term storage (−80°C). Among 5,565 CHS participants alive in 1992, plasma phospholipid fatty acids were measured in 3,736 participants (67%), including 3,238 randomly selected participants from among individuals with available blood samples and an additional 498 participants from a prior nested case-control study within CHS of incident heart disease.(27) Because these individuals were not a random sample of all CHS participants, all analyses accounted for within-cohort sampling using inverse-probability-of-sampling weights. All fatty acids, covariates, and metabolic outcomes were assessed similarly in all participants using the 1992 visit and blood sampling, except for dietary habits which were assessed at enrollment 3 years earlier (see below).

Fatty Acids

Fatty acid measurements were performed at the Fred Hutchinson Cancer Research Center, providing quantitative measurement of 45 fatty acids as percentage of total fatty acids. Plasma phospholipids represent a biomarker of longer-term (4–8 week) circulating fatty acids, with similar responses as levels in erythrocyte membranes.(28) Under blood storage conditions in CHS, we have observed no degradation, lipolysis, or oxidation after 10 years.(29) Total lipids were extracted from plasma using methods of Folch, and phospholipids separated from neutral lipids by one-dimensional thin-layer-chromatography. Fatty-acid-methyl-ester samples were prepared by direct transesterification using methods of Lepage and separated using gas-chromatography1 (5890 gas-chromatograph/flame-ionization-

detector, Agilent Technologies, Palo Alto, California; SP-2560 fused-silica 100m capillary column, Supelco, Belefonte, Pennsylvania; initial 160°C×16min, ramp 3.0°C/min to 240°C, hold 15min). Identification, precision, and accuracy were continuously evaluated using model mixtures of known fatty-acid-methyl-esters and established in-house controls, with identification confirmed at the US Department of Agriculture or for trans-fats by silver-ion thin-layer-chromatography. Laboratory coefficients of variation were 3.0% for trans-palmitoleate and <3% for most fatty acids. We assessed long-term reproducibility of trans-palmitoleate in a subset of 100 participants, that would capture both 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, comparable to within-individual correlations over time for other common risk factors such as blood pressure.(30)

Metabolic Measures and Covariates

Anthropometric measures were collected using standard procedures and equipment including weight, height, and waist circumference. Fasting blood lipids were measured according to U.S. Centers for Disease Control methods; low-density-lipoprotein (LDL)-cholesterol was calculated by the Friedewald equation, excluding hypertriglyceridemic individuals. Fasting glucose and insulin (Ektacham700 Analyzer, Eastman Kodak, Rochester, New York) were used to derive homeostasis-assessment-model of insulin resistance (glucose mg/dl×insulin mU/l)/405). Fibrinogen was measured using standard methods and C-reactive protein (CRP) using validated in-house high-sensitivity enzyme-linked-immunosorbent-assay. Standardized questionnaires assessed usual frequency and types of alcoholic beverages (wine, beer, liquor). Leisure-time activity was assessed by modified Minnesota Leisure-Time Activities questionnaire, evaluating frequency and duration of 15 different activities. Diet was assessed using a picture-sort food frequency questionnaire validated against six detailed 24-hour diet recalls spaced~1 month apart.(31)

Incident Diabetes

At annual study-clinic visits, participants brought all prescription medications taken in the previous two weeks; after 10 years, similar information was collected annually by telephone. Detailed medication data were obtained and recorded using computerized inventories, including drug names, doses, and frequencies coded according to prescription Medispan files. Prevalent and incident diabetes were defined by medication use and results of CHS blood testing. Prevalent diabetes was defined by use of insulin or hypoglycemic medication, fasting glucose≥7.0mmol/l (126mg/dl), or (among participants fasting<8 hrs; 1.7%) nonfasting glucose≥11.1 mmol/l (200mg/dl). Incident diabetes was defined by new use of insulin or hypoglycemic medication (assessed annually), fasting glucose≥7.0mmol/l (126mg/dl) (assessed in 1996), or 2-hour postchallenge glucose≥11.1mmol/l (200mg/dl) (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(32) with metabolic risk factors in a cohort of 327 generally healthy women, age=60.4±6.1 years, in the Nurses Health Study. Relationships were evaluated for high-density-lipoprotein (HDL)-cholesterol, total:HDL-cholesterol, interleukin-6, CRP, and hemoglobinA1c. Because many samples were nonfasting, triglycerides and LDL-cholesterol were not evaluated; and the number of subjects was too small to evaluate incident diabetes. Multivariable models were adjusted for age, smoking, physical activity, alcohol intake, family history, hypertension, hormone use, fasting status, and dietary carbohydrate, protein, polyunsaturated fats, saturated fat, dietary fiber, and total energy. We also evaluated correlations of trans-palmitoleate levels in plasma vs.

erythrocytes in Nurses Health Study (Spearman correlation[r]=0.74); and in phospholipids vs. triglycerides in CHS (n=104; r=0.54).

Statistical Analysis

We evaluated trans-palmitoleate in sex-specific quintiles as indicator variables and continuously per SD difference (0.05 percentage points). We assessed independent demographic and lifestyle factors associated with trans-palmitoleate using multivariable-adjusted linear regression with trans-palmitoleate as the dependent variable. Multivariable-adjusted relationships of trans-palmitoleate with metabolic risk factors (transformed for normality as appropriate) were evaluated using linear regression with trans-palmitoleate as the independent variable. Quintiles were evaluated as ordinal variables to assess trend and to assess effect modification using the Wald test for a multiplicative interaction term. We used Cox proportional-hazards 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, the latest date of adjudicated medication data. The proportional-hazards assumption was not rejected based on Schoenfeld residuals. To minimize potential confounding, covariates were selected based on biologic interest, being well-established risk factors for metabolic risk, or associations with exposures/outcomes in the present cohort. Missing covariates (most factors≤1.9%; dietary factors=7–10%) were imputed by best-subset regression using baseline age, sex, race, education, coronary heart disease (CHD), stroke, diabetes, smoking status, alcohol use, physical activity, body mass index (BMI), and, for nutritional factors, other relevant dietary variables; results using multiple imputation(33) or excluding missing values were similar. Analyses were performed using Stata10.1 (StataCorpLP, College Station, Texas), two-tailed-alpha=0.05.

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RESULTS

Trans-palmitoleate levels represented less than one percent of total fatty acids: mean±SD 0.18±0.05%, range=0.02, 0.55%. Trans-palmitoleate levels correlated strongly with fatty acid biomarkers of dairy fat consumption(34–36) such as 15:0 (r=0.64) and 17:0 (r=0.66), but weakly with trans-fats 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), and total trans-18:2 (r=0.07), consistent with dairy foods rather than industrially produced trans-fats being a major source of trans-palmitoleate. In bivariate (unadjusted) analyses (Table 1), trans-palmitoleate was associated with slightly older age, white race, and modestly less prevalent CHD. Trans-palmitoleate was 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 next elucidated and quantified the factors independently associated with circulating trans-palmitoleate levels in this large community-based cohort (Table 2). In multivariable analyses, older age, nonwhite race, and greater education were associated with higher levels, and female sex and prevalent CHD were associated with lower levels. Greater BMI and alcohol use were associated with slightly lower levels. Whole-fat dairy consumption had the strongest relationship, with 0.69 SD higher trans-palmitoleate levels among individuals consuming 15+ servings/week (2+ servings/day) versus ≤2 servings/week. Evaluating different dairy foods, this relationship appeared directly related to dairy fat content: it was strongest for whole milk (+0.49 higher SD of trans-palmitoleate per serving/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 multivariable-adjusted analyses, trans-palmitoleate levels significantly associated with several metabolic risk factors (Table 3). Higher trans-palmitoleate was associated with slightly lower BMI (across quintiles: −1.8%, P=0.058) and waist circumference (−1.8%, P=0.009). In multivariable-models further adjusted for adiposity, trans-palmitoleate was associated with slightly higher HDL-cholesterol (+1.9%, P=0.043), substantially lower triglycerides (−19.0%, P<0.001), lower total:HDL-cholesterol (−4.7%, P<0.001), and lower CRP (−13.8%, P=0.050), a marker of systemic inflammation related to metabolic syndrome and diabetes risk.(37,38) Trans-palmitoleate was also associated with fibrinogen, an acute phase reactant elevated by insulin resistance(39), but without dose-response: lower levels were seen only in the lowest quintile. Trans-palmitoleate was associated with lower fasting insulin (−13.3%, P<0.001) and insulin resistance (homeostasis-assessment-model: −16.7%, P<0.001). Trans-palmitoleate was unassociated with LDL-cholesterol, fasting glucose, or blood pressure. All relationships appeared similar by sex (not shown).

Among 2,985 participants free of prevalent diabetes, 304 new cases occurred during 27,866 person-years. In age- and gender-adjusted analyses, trans-palmitoleate was associated with lower risk of new-onset diabetes, including 2-fold lower risk in quintile 4 (HR=0.44, 95%CI=0.30–0.66) and 3-fold lower risk in quintile 5 (HR=0.36, 95%CI=0.23–0.57), versus quintile 1 (P-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). Lower risk appeared similar in men (extreme-quintile HR=0.29, 95%CI=0.11–0.74) and women (extreme-quintile HR=0.41, 95%CI=0.24–0.71) (P-interaction=0.87). When evaluated continuously per SD (0.05 percentage points of total fatty acids), each higher SD of trans-palmitoleate was associated with 28% lower risk of diabetes (multivariable-adjusted HR=0.72, 95%CI=0.61–0.86, P<0.001). To explore potential reverse causation (presence of subclinical disease altering trans-palmitoleate levels), we performed sensitivity analyses excluding 1,106 participants with fasting glucose≥5.56 mmol/l (100mg/dl) at baseline. Trans-palmitoleate remained inversely associated with incident diabetes (extreme-quintile HR=0.31, 95%CI=0.13–0.71).

Based on biologic considerations and present findings (Table 2), consumption of carbohydrate, protein, red meat, and dairy foods could each confound relations between trans-palmitoleate and incident diabetes, even in fully multivariable-adjusted analyses. We first evaluated whether these factors themselves were independently associated with incident diabetes. After multivariable-adjustment (covariates per Table 4) but excluding adjustment for trans-palmitoleate, neither consumption of carbohydrate (P=0.81), protein (P=0.88), red meat (P=0.69), nor low-fat dairy (P=0.98) were associated with incident diabetes. Conversely, greater whole-fat dairy consumption was associated with lower risk (across 6 categories, P-trend=0.024). However, when further adjusted for trans-palmitoleate levels, this association was attenuated and no longer significant (P-trend=0.162). In contrast, the relationship of trans-palmitoleate with diabetes risk was robust to adjustment for dairy consumption (Table 4).

These findings suggested that trans-palmitoleate, rather than consumption of specific foods, was the principal factor related to diabetes risk. However, we recognized that trans-palmitoleate was measured objectively, whereas food intakes were estimated by questionnaire. We evaluated whether other fatty acid dairy biomarkers (15:0, 17:0) related to diabetes risk as well as whether associations of trans-palmitoleate with diabetes risk persisted following adjustment for these biomarkers; we similarly evaluated trans-18:1n-7 (vaccenic acid) that is present in both dairy fats(40) and other food sources.(23) After

multivariable-adjustment (covariates per Table 4), neither 15:0 (HR=0.99, 95%CI=0.89–1.10), 17:0 (HR=0.98, 95%CI=0.85–1.13), nor vaccenic acid (HR=0.97, 95% CI=0.93–1.01) were associated with diabetes risk (HR's evaluated per 0.05 percentage points of total fatty acids, corresponding to 1.0, 0.7, and 0.3 SD differences, respectively). However, following simultaneous adjustment for levels of 15:0, 17:0, and vaccenic acid, trans-palmitoleate remained inversely associated with new-onset diabetes (extreme-quintile HR=0.43, 95%CI=0.25–0.75, P-trend<0.001).

In our separate validation cohort, higher erythrocyte trans-palmitoleate was associated with lower interleukin-6 (multivariable-adjusted levels across quartiles: 2.3 vs. 1.8 pg/ml, P-trend=0.020) and CRP (3.3 vs. 2.1 mg/l, P-trend=0.020) and trends toward lower total:HDL-cholesterol (4.21 vs. 3.87, P-trend=0.099) and hemoglobinA1c (5.92 vs. 5.66 percent, P-trend=0.056). In similar analyses, higher plasma trans-palmitoleate was associated with higher HDL-cholesterol (1.46 vs. 1.60 mmol/l [56.5 vs. 61.7 mg/dl], P-trend=0.033), lower total:HDL-cholesterol (4.28 vs. 3.63, P-trend<0.001), and lower hemoglobinA1c (5.88 vs. 5.64 percent, P-trend=0.028).

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, independent of this, with higher HDL-cholesterol; lower triglycerides, total:HDL-cholesterol, and insulin resistance; and substantially lower onset of diabetes, with nearly 3-fold lower risk across quintiles. The magnitude and robustness of these relationships were notable. 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, supporting specificity of trans-palmitoleate, rather than dairy consumption per se, as related to 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.

Based on our English-language MEDLINE search through June 2010, experimental effects of trans-palmitoleate on metabolic risk have not been reported to directly evaluate mechanisms of benefit. In the present work, trans-palmitoleate was unassociated with LDL-cholesterol or blood pressure levels, suggesting 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 we observed when adipose tissue production of cis-palmitoleate was experimentally upregulated.(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 non-hepatic sources might provide counter-regulatory feedback against hepatic fat synthesis. Considerable experimental evidence suggests that increased hepatic fat synthesis contributes to nonalcoholic steatohepatitis and associated insulin resistance.(41–43) We wonder whether trans-palmitoleate, as an exogenous, nonhepatic source of palmitoleate, could partly suppress hepatic fat synthesis or have other beneficial physiologic effects (e.g., augmenting skeletal muscle glucose uptake) by mimicking or competing with pathways of effect of either cis-palmitoleate (shared molecular structure but different bond configuration) or 16:0 (different bond structure but similar stereochemical shape). For example, this could mimic a putative

counterregulatory role of adipose-produced cis-palmitoleate that has been largely lost with typical modern diets, whose high carbohydrates and excess energy are key stimulators of hepatic fat synthesis.(28,44–48)

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,(49) with compensatory upregulation of adipose tissue genes involved in fat synthesis.(50) Although no prior studies have reported on trans-palmitoleate and metabolic risk, a small study in patients with established CHD (n=191) observed a nonsignificant inverse trend between platelet trans-palmitoleate levels and coronary atherosclerosis (p=0.12), whereas other trans-fats were associated with greater atherosclerosis.(51) In light of our present observations, 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; indeed, three cohorts have observed nonsignificant trends toward inverse associations.(22) These finding remain unexplained, as major ruminant trans-fats (predominantly trans-18:1 isomers) appear to adversely affect blood cholesterol levels similar to industrial trans-fats at equivalent doses.(52) Our findings suggest trans-palmitoleate, a fatty acid relatively unique to ruminant foods,(23) could at least partly offset adverse effects of other trans-fats in ruminant foods. Additionally, multiple large cohorts have recently reported inverse associations between dairy consumption and risk of obesity, metabolic risk factors, or type2 diabetes,(24,25,53–61) without consistent differences for different types of dairy foods.(55–59) Vaccenic acid and calcium were proposed mediators of such benefits, but vaccenic acid and its metabolites (e.g., conjugated linoleic acid) produced disappointingly adverse effects on blood lipids and insulin resistance,(62–65) and clinical studies evaluating dairy calcium found small or no metabolic benefits.(66,67) Our findings support potential metabolic benefits of dairy consumption and suggest that trans-palmitoleate may be a candidate to mediate these effects. Our results also suggest that efforts to promote exclusive consumption of low-fat and nonfat dairy products, that would lower population exposure to trans-palmitoleate, may be premature until the mediators of 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, providing a community-based sample and increasing generalizability. A number of demographic, lifestyle, and dietary covariates were available for multivariable-adjustment, minimizing residual confounding.

Potential limitations should be considered. Associations with metabolic risk factors were cross-sectional, limiting assessment of temporality. However, prospective analyses of diabetes incidence were strongly supportive. Measurement error and biologic variability were present in exposures and covariates, which could bias results in unpredictable directions. Although we adjusted for major metabolic risk factors, residual confounding by unmeasured or imperfectly measured factors may be present. Conversely, magnitudes of multivariable-adjusted findings, including nearly 3-fold lower diabetes incidence, renders it improbable that residual confounding fully accounts for these relationships. Additionally, protective associations were independent of estimated dairy consumption and other dairy fatty acid biomarkers. Blood glucose was not measured annually, and diabetes incidence

might be underestimated. We evaluated several relationships and metabolic risk factors as outcomes, and thus P-values should be considered as a guide.

Our results demonstrate an inverse relationship between trans-palmitoleate and metabolic risk factors and diabetes incidence. The small differences in trans-palmitoleate levels raise questions whether this is the active compound versus a marker for some other unknown protective constituent of dairy and/or other ruminant foods, but also suggest that, if causal, this fatty acid has potential as a target 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 need for additional detailed experimental and clinical investigation, including animal-experiments and metabolic feeding studies, to more fully assess the potential health effects of trans-palmitoleate.

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Table 1

Baseline Characteristics According to Plasma Phospholipid Trans-Palmitoleic Acid in 3,736 US Adults.

	Sex-Specific Quintiles of Trans-Palmitoleic Acid				
	I	**II**	**III**	**IV**	**V**
% total fatty acids	**0.12±0.02**	**0.16±0.01**	**0.18±0.01**	**0.21±0.01**	**0.26±0.03**
n	**(n=767)**	**(n=738)**	**(n=744)**	**(n=748)**	**(n=739)**
Age, years	75±5	75±5	75±5	76±5	76±5
Sex, % male	43	47	45	46	46
Race, % white	81	84	86	90	91
Education > high school, %	42	43	46	47	46
Current smoking, %	9	6	8	7	10
Coronary heart disease, %	26	27	26	23	19
Diabetes mellitus, %	17	18	19	17	13
Exercise intensity moderate+, %	44	42	45	46	44
Leisure-time activity, kcal/wk	1084±1422	1056±1433	1170±1475	1063±1471	943±1269
Alcohol, drinks/week	3.7±10.3	2.2±4.9	1.8±4.6	1.6±4.1	1.3±3.9
Total fat, % energy	30.7±6	31.6±6	32.0±6	32.8±5	32.9±6
Carbohydrate, % energy	53.8±8	52.8±8	52.4±7	51.6±7	51.5±7
Whole-fat dairy, servings/wk †	5.7±4.3	6.5±4.8	6.7±4.5	7.9±4.9	8.7±5.4
Low-fat dairy, servings/wk †	4.1±3.7	4.3±3.9	3.8±3.4	3.8±3.4	3.6±3.7
Red meat, 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 that was assessed 3 years earlier. Values are mean±SD (continuous variables) or percent (categorical variables).

†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.

Table 2

Multivariable-Adjusted Relationships of Demographic, Clinical and Lifestyle Factors with Plasma Phospholipid Trans-Palmitoleic Acid in 3,736 US Adults.

		SD Difference (95% CI) in Trans-Palmitoleic Acid *	P value
Age, each 5 years		0.08 (0.04, 0.12)	<0.001
Sex	Male	reference	
	Female	−0.25 (−0.34, −0.16)	<0.001
Race	White	reference	
	Nonwhite	0.33 (0.19, 0.48)	<0.001
Education	Less than high school	reference	
	High school graduate	0.07 (−0.04, 0.18)	0.22
	Some college	0.14 (0.02, 0.27)	0.019
	College graduate	0.16 (0.03, 0.29)	0.017
Prevalent diabetes	No	reference	
	Yes	−0.03 (−0.14, 0.09)	0.63
Prevalent CHD	No	reference	
	Yes	−0.14 (−0.23, −0.04)	0.007
Smoking	Never	reference	
	Former	−0.04 (−0.13, 0.05)	0.41
	Current	0.09 (−0.06, 0.24)	0.26
Body mass index, each kg/m2		−0.01 (−0.02, −0.00)	0.004
Leisure-time activity, each 500 kcal/wk		−0.01 (−0.02, 0.01)	0.28
Alcohol, each drink/week		−0.03 (−0.04, −0.02)	<0.001
Carbohydrate, each higher 5% energy replacing fat		−0.05 (−0.08, −0.01)	0.014
Protein, each higher 5% energy replacing fat		−0.08 (−0.16, 0.00)	0.045
Whole-fat dairy	0–2 servings/wk	reference	
	3–5	0.15 (0.01, 0.28)	0.036
	6–8	0.21 (0.08, 0.34)	0.001
	9–11	0.41 (0.27, 0.55)	<0.001
	12–14	0.40 (0.24, 0.56)	<0.001
	15+	0.69 (0.49, 0.89)	<0.001
Low-fat dairy, each serving/d		−0.01 (−0.03, −0.00)	0.034
Red meat, each serving/d		0.14 (−0.00, 0.27)	0.058

* Values are multivariable-adjusted associations of each factor with trans-palmitoleic acid levels, evaluated per 1 SD (0.05 percentage points of total fatty acids), adjusted for each of the variables in the table 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 that was assessed 3 years earlier.

CHD=coronary heart disease.

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Table 3

Multivariable-Adjusted Relationships of Plasma Phospholipid Trans-Palmitoleic Acid with Metabolic Risk Factors in 3,736 US Adults.

	Quintiles of Trans-Palmitoleic Acid					P for Trend
	I	II	III	IV	V	
% total fatty acids, median	**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, mg/dl	126	128	127	126	129	0.63
HDL-cholesterol, mg/dl	53.0	52.0	52.1	53.7	54.0	0.043
Triglycerides, mg/dl	147	134†	128‡	120‡	119‡	<0.001
Total/HDL-cholesterol ratio	4.3	4.3	4.2	4.1†	4.1†	<0.001
Inflammation						
C-reactive protein, mg/l	2.9	2.8	2.6	2.7	2.5	0.050
Fibrinogen, mg/dl	316	327†	329†	332‡	328†	0.006
Glucose-Insulin Homeostasis						
Fasting glucose, mg/dl	104	105	103	103	103	0.103
Fasting insulin, mU/l	11.3	11.0	10.7	10.2†	9.8‡	<0.001
HOMA-IR, units	3.0	2.9	2.8*	2.7†	2.5‡	<0.001
Blood Pressure						
Systolic, mm Hg	137	136	136	136	136	0.48
Diastolic, mm Hg	71	71	70	71	71	0.98

Values are adjusted means (transformed to approximate normality for analyses and re-transformed as necessary), adjusted for age (years), sex, race (white/nonwhite), education (<high school, high school, some college, college graduate), enrollment site (4 sites), smoking (never, former, current), diabetes (yes/no), coronary heart disease (yes/no), physical activity (kcal/wk), alcohol use (6 categories), and consumption of carbohydrate (% energy), protein (% energy), red meat (servings/week), whole-fat dairy foods (6 categories), low-fat dairy foods (5 categories), and total energy (kcal/d). Results for blood lipid, inflammatory, glucose-insulin, and blood pressure measures are also adjusted for body mass index (kg/m2) and waist circumference (cm).

*P<0.05,

†P<0.01,

‡P<0.001 compared with quintile 1.

To convert LDL-cholesterol and HDL-cholesterol from mg/dl to mmol/l, divide by 38.67. To convert triglycerides from mg/dl to mmol/l, divide by 88.57. To convert glucose from mg/dl to mmol/l, divide by 18.0.

HOMA-IR=homeostasis-assessment-model of insulin resistance.

Table 4

Incidence of Diabetes Mellitus Between 1992 and 2006 According to Plasma Phospholipid Trans-Palmitoleic Acid in 3,736 US Adults without Prevalent Diabetes at Baseline.

	Quintiles of Trans-Palmitoleic Acid					P for trend
	I	II	III	IV	V	
Person-years of followup	5,595	5,435	5,556	5,811	5,469	
No. of incident cases	93	68	59	46	38	
Hazard ratio (95% CI)						
Age- and gender-adjusted	1.0	0.84	0.88	0.44	0.36	<0.001
	(reference)	(0.57–1.22)	(0.59–1.31)	(0.30–0.66)	(0.23–0.57)	
Multivariable-adjusted	1.0	0.79	0.89	0.41	0.38	<0.001
	(reference)	(0.54–1.15)	(0.58–1.33)	(0.27–0.64)	(0.24–0.62)	

Incident diabetes was defined by new use of insulin or oral hypoglycemic medication, fasting glucose ≥7.0 mmol/l (126 mg/dl), or 2-hour postchallenge glucose ≥11.1 mmol/l (200 mg/dl), excluding individuals with prevalent diabetes at baseline. Multivariable model adjusted for age (years), gender (male/female), race (white/nonwhite), education (<high school, high school, some college, college graduate), enrollment site (4 sites), smoking (never, former, current), body mass index (kg/m2), waist circumference (cm), coronary heart disease (yes/no), physical activity (kcal/wk), alcohol use (6 categories), and consumption of carbohydrate (% energy), protein (% energy), red meat (servings/week), whole-fat dairy foods (6 categories), low-fat dairy foods (5 categories), and total energy (kcal/d).

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