trans-Palmitoleic acid, other dairy fat biomarkers, and incident diabetes: the Multi-Ethnic Study of Atherosclerosis (MESA)[1–3]

Dariush Mozaffarian, Marcia C de Oliveira Otto, Rozenn N Lemaitre, Amanda M Fretts, Gokhan Hotamisligil, Michael Y Tsai, David S Siscovick, and Jennifer A Nettleton

ABSTRACT

Background: Dairy consumption is linked to a lower risk of type 2 diabetes, but constituents responsible for this relation are not established. Emerging evidence suggests that *trans*-palmitoleate (*trans* 16:1n–7), a fatty acid in dairy and also partially hydrogenated oils, may be associated with a more favorable metabolic profile and less incident diabetes.

Objective: We investigated the association of *trans*-palmitoleate with metabolic risk and incident diabetes in a multiethnic US cohort.

Design: Phospholipid fatty acids and metabolic risk factors were measured in 2000–2002 among 2617 adults in the Multi-Ethnic Study of Atherosclerosis (MESA), a cohort of white, black, Hispanic, and Chinese Americans. In 2281 participants free of baseline diabetes, we also prospectively assessed the risk of new-onset diabetes (205 cases) from baseline to 2005–2007.

Results: *trans*-Palmitoleate concentrations correlated positively with self-reported consumption of whole-fat dairy, butter, margarine, and baked desserts and with other circulating biomarkers of both dairy fat and partially hydrogenated oil consumption, which suggested mixed dietary sources. After multivariable adjustment, *trans*-palmitoleate concentrations were associated with higher LDL cholesterol (quintile 5 compared with quintile 1: +6.4%; *P*-trend = 0.005), lower triglycerides (−19.1%; *P*-trend < 0.001), lower fasting insulin (−9.1%; *P*-trend = 0.002), and lower systolic blood pressure (−2.4 mm Hg; *P*-trend = 0.01). In prospective analyses, *trans*-palmitoleate was independently associated with lower incident diabetes (*P*-trend = 0.02), including a 48% lower risk in quintile 5 compared with quintile 1 (HR: 0.52; 95% CI: 0.32, 0.85). All findings were similar between men and women and between different race-ethnic subgroups.

Conclusions: Circulating *trans*-palmitoleate is associated with higher LDL cholesterol but also with lower triglycerides, fasting insulin, blood pressure, and incident diabetes in a multiethnic US cohort. Our findings support the need for further experimental and dietary intervention studies that target circulating *trans*-palmitoleate. The MESA trial was registered at clinicaltrials.gov as NCT00005487. *Am J Clin Nutr* 2013;97:854–61.

INTRODUCTION

Dairy consumption has been linked to less metabolic dysfunction and lower incidence of type 2 diabetes in observational studies (1), but potential mechanisms for such benefits remain poorly understood. Some studies suggest that a lower diabetes risk may be more closely linked to consumption of yogurt and low-fat milk, rather than whole-fat dairy, but results have been conflicting (2–4), and no studies have evaluated dairy fat per se. Most studies have also relied on self-reported dietary estimates, which may be biased by measurement errors due to participants' imperfect recall, selective reporting, or misreporting of different types of dairy foods.

We recently reported that plasma phospholipid *trans*-palmitoleic acid (*trans* 16:1n–7), an objective circulating fatty acid biomarker contained in both dairy fat and partially hydrogenated oils, was cross-sectionally associated with an improved metabolic profile and prospectively associated with a substantially lower incidence of type 2 diabetes in a cohort of older US adults (5). Compared with the lowest quintile, individuals in the highest quintile of plasma phospholipid *trans*-palmitoleic acid had a more than 2-fold lower risk of incident diabetes (5). In addition to its dietary sources, this fatty acid is also interesting given its isomeric similarity to its sister fatty acid, *cis*-palmitoleic acid (*cis* 16:1n–7), which in animal models and in in vitro experiments improves peripheral insulin resistance, reduces metabolic dysregulation, and inhibits hepatic de novo lipogenesis (6–10).

These findings suggested that dairy fat consumption, and possibly *trans*-palmitoleate specifically, could play a role in the observed metabolic benefits of dairy consumption, with pathways potentially related to modulation of insulin resistance and regulation of hepatic fat synthesis. In our original report, we replicated

[1] From the Division of Cardiovascular Medicine and Channing Laboratory, Brigham and Women's Hospital and Harvard Medical School, Boston, MA (DM); the Departments of Epidemiology (DM and MCdOO), Nutrition (DM and GH), and Genetics and Complex Diseases (GH), Harvard School of Public Health, Boston, MA; the Cardiovascular Health Research Unit, Department of Medicine, University of Washington, Seattle, WA (RNL, AMF, and DSS); the Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN (MYT); and the Division of Epidemiology, Human Genetics, and Environmental Sciences, University of Texas Health Science Center at Houston, Houston, TX (JAN).

[2] Supported by the National Heart, Lung, and Blood Institute, NIH (R01-HL085710; to DM, RNL, AMF, and DSS); the National Institute of Diabetes and Digestive and Kidney Diseases, NIH (K01-DK082729; to JAN); and the Harvard Bunge Fellowship Fund, an unrestricted educational grant from Bunge Global Innovation, LLC (to MCdOO). MESA is conducted and supported by contracts N01-HC-95159 through N01-HC-95169 and RR-024156 from the National Heart, Lung, and Blood Institute.

[3] Address correspondence to D Mozaffarian, Harvard School of Public Health, 665 Huntington Avenue, Building 2-319, Boston, MA 02115. E-mail: dmozaffa@hsph.harvard.edu.

Received June 22, 2012. Accepted for publication January 7, 2013.

First published online February 13, 2013; doi: 10.3945/ajcn.112.045468.

cross-sectional associations of higher *trans*-palmitoleate with less atherogenic dyslipidemia and better glucose-insulin homeostasis among women in a second independent cohort, but case numbers were too small to evaluate findings for incident diabetes (5).

Given the unexplained inverse associations of dairy foods with diabetes risk and the robust inverse association of *trans*-palmitoleate with incident diabetes in one prior study, we investigated whether phospholipid *trans*-palmitoleate as well as other dairy fat biomarkers were prospectively associated with a lower incidence of diabetes in the Multi-Ethnic Study of Atherosclerosis (MESA)[4], a cohort that included diverse race-ethnic and dietary variation (11).

SUBJECTS AND METHODS

Design and population

The MESA is a prospective cohort study designed to investigate risk factors associated with subclinical cardiovascular disease in US adults across a range of races and ethnicities. The study design has been described in detail (12). Briefly, 6814 adults aged 45–84 y and free of clinical cardiovascular disease at baseline were recruited in 2000–2002 from 6 US communities: Baltimore City and County, MD; Chicago, IL; Forsyth County, NC; New York, NY; Los Angeles County, CA; and St Paul, MN. Cohort examinations were conducted in 2000–2002 (baseline/exam 1), 2002–2003 (exam 2), 2004–2005 (exam 3), and 2005–2007 (exam 4). Protocols were approved by local institutional review boards, and all participants provided written informed consent. An ancillary study measured plasma phospholipid fatty acids in a random sample of 2855 MESA participants equally distributed across each race-ethnic group. After excluding 218 participants whose dietary data did not meet quality-control checks (13) and 20 with missing covariate data, 2617 participants were included in the present analysis.

Measurement of fatty acids

At baseline, fasting blood was collected, processed, and stored at −70°C by using standardized protocols (12, 14). Plasma phospholipid fatty acids were measured in these stored samples by using thin-layer chromatography separation and gas chromatography quantification (15) (*see* Supplementary Materials under "Supplemental data" in the online issue for details). These methods quantified the concentrations of 27 individual fatty acids as percentages of total fatty acids. For this analysis, our primary exposure of interest was *trans* 16:1n–7. To elucidate whether findings were specific for *trans*-palmitoleate, which could feasibly be bioactive, or were related to dairy fat in general, we also evaluated 14:0 and 15:0, SFA biomarkers of dairy consumption (16–18), for their independent and mutually adjusted associations with incident diabetes. We recognized that 14:0 can also by synthesized by de novo lipogenesis, potentially confounding its associations. The MESA did not measure 17:0, another potential dairy biomarker. For each fatty acid, the automated (computer software) limit of detection was 0.03%. All peaks <0.03% were manually reviewed and quantified, with a manual limit of detection of 0.01%. Laboratory CVs across a range of control sample concentrations (very low to high) were 29.5% for *trans* 16:1n–7, 11.6% for 14:0, and 14.5% for 15:0. Laboratory CVs excluding the lowest (≤0.05%) control sample concentrations were 10.9% for *trans* 16:1n–7, 5.2% for 14:0, and 7.7% for 15:0.

Anthropometric measurements, metabolic risk markers, and diet

At each study examination, a combination of self-administered and interviewer-administered questionnaires assessed demographic characteristics, education, medication use, smoking history, physical activity, and dietary habits. Concentrations of total and HDL cholesterol, triglycerides, insulin, and glucose were centrally measured (Collaborative Studies Clinical Laboratory, Fairview-University Medical Center, Minneapolis, MN) by using standardized methods (Roche Diagnostics reagents), and LDL cholesterol was calculated from the Friedewald equation, excluding those subjects with triglycerides ≥400 mg/dL (n = 41) (19). Resting seated blood pressure was measured 3 times (Dinamap Model Pro 100 automated oscillometer; Critikon); we used the average of the last 2 measures. Weight, height, and waist circumference were measured by trained personnel by using standardized instruments, and BMI was calculated as weight (to the nearest 0.45 kg) divided by height (to the nearest 0.1 cm) squared. Medications were assessed by both self-report and medication inventory. At baseline, usual dietary intake over the past year was assessed by using a 120-item food-frequency questionnaire (13, 20). The questionnaire was developed in the validated Block format and was patterned after the questionnaire used in the Insulin Resistance Atherosclerosis Study and validated in non-Hispanic whites, African Americans, and Hispanics; it was further modified to include specific Chinese foods and culinary practices (13, 20).

Diagnosis of incident diabetes

Fasting serum glucose was measured at each examination by rate reflectance spectrophotometry by using thin-film adaptation of the glucose oxidase method (Vitros analyzer; Johnson & Johnson Clinical Diagnostics). For longitudinal analysis of incident diabetes, we excluded 336 individuals with prevalent diabetes at baseline. Incident diabetes was diagnosed on the basis of new fasting glucose ≥126 mg/dL or the new use of insulin or oral hypoglycemic medications, each assessed at study clinic examinations approximately every 2 y during follow-up.

Statistical analysis

Intercorrelations between plasma phospholipid fatty acids and between fatty acids and foods were evaluated by using Spearman's correlation (r). Potential independent determinants of *trans*-palmitoleate concentrations were evaluated by using multivariable-adjusted linear regression with *trans*-palmitoleate as the dependent variable. To assess associations with metabolic risk factors and incident diabetes, *trans*-palmitoleate concentrations were evaluated as independent indicator variables in quintiles and continuously per SD. We used linear regression with robust variance estimators to assess associations of *trans*-palmitoleate with metabolic risk factors. For longitudinal analyses, Cox proportional hazards estimated the HR of incident diabetes, with time at-risk until first diagnosis, death, or administrative

[4]Abbreviations used: CHS, Cardiovascular Health Study; MESA, Multi-Ethnic Study of Atherosclerosis; NHS, Nurses' Health Study.

censoring at the latest date of adjudicated follow-up (2005–2007). 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 in the current cohort. We determined the potential for effect modification by age, race-ethnicity, adiposity, sex, and alcohol use by evaluating the significance of multiplicative interaction terms with the use of likelihood ratio tests. To obtain the most robust estimates of association with incident diabetes, we pooled the present MESA findings with our prior results from the Cardiovascular Health Study (CHS) (5) by using cohort-specific indicator quintiles as well as continuously using inverse-variance fixed-effects meta-analysis (21), and we tested dose-response across all quintiles in both cohorts by using generalized least-squares trend estimation (22). Analyses were performed by using SAS, version 9.2 (SAS Institute) and Stata12.1 (StataCorp) (2-tailed $\alpha = 0.05$).

RESULTS

At baseline, the average (mean ± SD) age was 61.7 ± 10.2 y, 53.3% of subjects were female, and race-ethnicity was fairly evenly divided among white ($n = 674$), black ($n = 607$), Hispanic ($n = 640$), and Chinese ($n = 696$) participants (**Table 1**). Consistent with our previously observed findings in the CHS (5), *trans*-palmitoleate concentrations in the MESA represented <1% of total fatty acids (mean ± SD: 0.06 ± 0.03%; range: 0.01–0.24%). Unadjusted *trans*-palmitoleate concentrations were higher among white, black, and Hispanic participants (means ranged from 0.06% to 0.07%) than among Chinese participants (mean: 0.04%), which is consistent with a higher average consumption of whole-fat dairy foods in the former groups (means: 5.8–6.2 servings/wk) compared with the latter group (mean: 2.8 servings/wk).

trans-Palmitoleate concentrations correlated with 15:0 (Spearman's $r = 0.33$, $P < 0.001$), a biomarker of dairy fat consumption, but not with 14:0 ($r = 0.02$, $P = 0.63$), a biomarker of dairy fat and endogenous synthesis; and they correlated with total *trans*18:1 (sum of n–6 through n–9*trans*18:1 isomers) ($r = 0.70$, $P < 0.001$), which are *trans* fats present in both dairy fats and partially hydrogenated oils (23). Consistent with these biomarker findings, *trans*-palmitoleate concentrations also correlated positively with the following: self-reported intakes of sources of dairy fat, such as whole-fat dairy and butter; partially hydrogenated oils, such as margarine, french fries, and fried snacks; and either dairy fat or partially hydrogenated oils, such as cakes, cookies, and pies (**Table 2**). Concentrations of *trans*18:1 similarly correlated positively with sources of both partially hydrogenated oils and dairy fat (not shown). Concentrations of 14:0 and 15:0 correlated with intakes of both whole-fat and low-fat dairy products. *trans*-Palmitoleate concentrations also inversely correlated with alcohol consumption (partial $r = -0.13$, $P < 0.001$), a stimulant of hepatic de novo lipogenesis and hepatic *cis*-palmitoleate production (24).

In multivariable-adjusted analyses, *trans*-palmitoleate concentrations were significantly associated with several metabolic risk factors, including higher LDL cholesterol but also lower triglycerides, lower fasting insulin, and lower blood pressure (**Table 3**). *trans*-Palmitoleate was not significantly associated

TABLE 1
Baseline characteristics of 2617 participants in the MESA, 2000–2002[1]

	Whites ($n = 674$)	Blacks ($n = 607$)	Hispanics ($n = 640$)	Chinese ($n = 696$)	All ($n = 2617$)
Phospholipid *trans*-palmitoleate (% of total fatty acids)	0.07 ± 0.03[2]	0.07 ± 0.03	0.06 ± 0.02	0.04 ± 0.02	0.06 ± 0.03
Phospholipid 14:0 (% of total fatty acids)	0.29 ± 0.08	0.23 ± 0.08	0.29 ± 0.08	0.23 ± 0.07	0.26 ± 0.08
Phospholipid 15:0 (% of total fatty acids)	0.20 ± 0.06	0.15 ± 0.04	0.18 ± 0.05	0.14 ± 0.03	0.17 ± 0.05
Age (y)	61.5 ± 10.5	61.7 ± 9.9	61.2 ± 10.1	62.3 ± 10.3	61.7 ± 10.2
Sex (% male)	46.4	45.6	46.4	48.0	46.7
Education >high school (%)	95.7	87.0	54.2	76.0	78.3
Current smoking (%)	14.8	19.0	13.6	5.2	12.9
BMI (kg/m^2)	27.8 ± 5.2	30 ± 5.6	29.5 ± 5	24 ± 3.3	27.7 ± 5.4
Waist circumference (cm)	97.6 ± 14.9	100.7 ± 14.1	100.5 ± 12.8	87.1 ± 9.9	96.3 ± 14.2
Prevalent diabetes (%)	5.9	16.5	16.9	12.6	12.8
Leisure-time activity (MET-min/wk)	2685 ± 3028	2808 ± 3352	2031 ± 2471	1718 ± 1939	2296 ± 2767
Total energy (kcal/d)	1564 ± 689	1608 ± 926	1607 ± 876	1167 ± 570	1479 ± 793
Total fat (% of energy)	32.4 ± 7.6	32.7 ± 7.2	30.0 ± 6.6	27.6 ± 5.5	30.6 ± 7.1
Saturated fat (% of energy)	10.9 ± 3.4	10.3 ± 3	10.3 ± 3.1	7.9 ± 2.3	9.8 ± 3.2
Monounsaturated fat (% of energy)	12.4 ± 3.1	12.8 ± 3.1	11.6 ± 2.8	10.7 ± 2.3	11.8 ± 2.9
Polyunsaturated fat (% of energy)	6.4 ± 1.9	6.7 ± 1.9	5.5 ± 1.7	6.3 ± 1.6	6.2 ± 1.8
Carbohydrate (% of energy)	50.4 ± 9.7	52.5 ± 9.4	54.7 ± 8.5	55.6 ± 7.7	53.3 ± 9.1
Protein (% of energy)	15.7 ± 3.4	15.5 ± 3.3	16.0 ± 3.2	17.9 ± 3.2	16.3 ± 3.4
Whole-fat dairy (servings/wk)[3]	6.7 ± 9.4	6.0 ± 7.6	6.4 ± 7.7	2.8 ± 5.7	5.5 ± 7.9
Low-fat dairy (servings/wk)[3]	7.8 ± 10.7	4.2 ± 7.2	7.0 ± 10.5	4.3 ± 7.3	5.9 ± 9.2
Red meat (servings/wk)	3.9 ± 3.5	4.2 ± 4.3	3.8 ± 4.2	3.6 ± 2.8	3.9 ± 3.7
Alcohol (drinks/wk)	4.1 ± 6.8	1.6 ± 3.5	1.5 ± 4.1	0.6 ± 2.5	1.9 ± 4.7

[1] MESA, Multi-Ethnic Study of Atherosclerosis; MET, metabolic equivalent.
[2] Mean ± SD (all such values).
[3] Whole-fat dairy included whole milk, regular cheese, cottage or ricotta cheese, whole-fat yogurt, and ice cream. Low-fat dairy included 2% milk, 1% or skim milk, and low-fat yogurt.

TABLE 2
Multivariable-adjusted correlations of self-reported dietary habits with plasma phospholipid *trans*-palmitoleate, 14:0, and 15:0 in the MESA, 2000–2002[1]

	trans-Palmitoleate	14:0	15:0
Whole-fat dairy	0.15	0.10	0.15
Cottage or ricotta cheese[2]	0.11	0.12	0.15
Other cheese[2]	0.17	0.13	0.21
Whole milk	—	—	0.07
Whole-fat yogurt	—	—	—
Ice cream	0.12	0.06	0.10
Low-fat dairy	—	0.09	0.14
2% milk	—	—	—
1%/skim milk	—	0.07	0.07
Low-fat yogurt	0.07	0.05	0.06
Butter	0.14	0.07	0.13
Cakes, cookies, or pies	0.18	0.06	0.09
Margarine	0.22	—	—
French fries	0.20	—	—
Fried snacks[3]	0.07	—	−0.04

[1] Values are partial correlation coefficients, adjusted for age, sex, race-ethnicity, field center, and energy intake. Only correlations with $P < 0.05$ are shown. We evaluated major potential food sources of dairy fat and partially hydrogenated oils. All correlations were evaluated for the total population, including persons not consuming these foods. MESA, Multi-Ethnic Study of Atherosclerosis.

[2] Although both whole-fat and low-fat versions of many cheeses are sold, even low-fat cheeses generally contain more dairy fat than either whole milk or whole-fat yogurt. Thus, all cheeses were included in the category of whole-fat dairy.

[3] Includes potato, corn, or tortilla chips; crackers; pretzels; and popcorn.

with HDL cholesterol, total to HDL-cholesterol ratio, or fasting glucose. All results appeared to be similar in each race-ethnic group (not shown). *trans*-Palmitoleate concentrations were not cross-sectionally associated with concentrations of C-reactive protein, IL-6, or homocysteine (not shown). Findings were not appreciably altered by additional adjustment for other dietary factors, including consumption of fruit, vegetables, and whole grains, or for use of lipid-lowering and antihypertensive medication (not shown). The exception was the association with LDL cholesterol, which was attenuated by adjustment for medication use (mean ± SE concentrations across quintiles: 113.2 ± 1.3, 118.2 ± 1.1, 119.0 ± 1.6, 118.2 ± 1.4, and 118.2 ± 1.7 mg/dL; *P*-trend = 0.051).

During up to 7 y of follow-up, 205 new cases of incident diabetes were diagnosed. Incidence rates per 10,000 person-years were 153 in whites, 223 in blacks, 246 in Hispanics, and 148 in Chinese participants. After adjustment for potential confounders including age, sex, race-ethnicity, field center, education, smoking, alcohol, physical activity, BMI, and waist circumference, higher *trans*-palmitoleate concentrations were associated with a lower risk of new-onset diabetes (*P*-trend = 0.02), with a 2-fold lower risk in the highest quintile (HR: 0.52; 95% CI: 0.32, 0.84) (**Table 4**). Further adjustment for dietary factors including consumption of whole-fat dairy, low-fat dairy, red meat, and total energy did not appreciably alter these results. Further adjustment for other covariates, such as consumption of margarine, butter, cakes, cookies, pies, salty snacks, fried potatoes, fruit, vegetables, or whole grains also did not alter these results (not shown).

When evaluated continuously per SD (0.03 percentage points of total fatty acids), each higher SD of *trans*-palmitoleate was associated with a 20% lower risk of diabetes (HR: 0.80; 95% CI: 0.66, 0.96; $P = 0.02$; covariates as in model 2, Table 4). Lower risk appeared to be generally similar by race-ethnicity, although CIs were broader due to limited statistical power in each subgroup: the multivariable-adjusted HR (95% CI) per each higher SD was 0.82 (0.55, 1.23) in whites, 0.79 (0.55, 1.12) in blacks, 0.73 (0.51, 1.05) in Hispanics, and 0.88 (0.53, 1.45) in Chinese. HRs were also similar when analyzed separately in men [0.88 (0.67, 1.15)] or women [0.75 (0.58, 0.98)]. There was little

TABLE 3
Multivariable-adjusted cross-sectional relations of plasma phospholipid *trans*-palmitoleic acid with metabolic risk factors among 2617 US adults in the MESA[1]

		Quintile of *trans*-palmitoleic acid					
	n[2]	1	2	3	4	5	*P*-trend[3]
trans-Palmitoleic acid (% of fatty acids)[4]	—	0.03 (0.01–0.03)	0.04 (0.04)	0.05 (0.05)	0.07 (0.06–0.07)	0.10 (0.08–0.24)	
LDL cholesterol (mg/dL)	2575	112.3 ± 1.3[5]	116.8 ± 1.7	119.3 ± 1.6	118.7 ± 1.3	119.2 ± 1.4	0.005
HDL cholesterol (mg/dL)	2616	50.7 ± 0.5	52.0 ± 0.7	50.5 ± 0.6	50.5 ± 0.5	50.1 ± 0.6	0.18
Triglycerides (mg/dL)	2616	148.5 ± 3.5	138.5 ± 4.1	135.0 ± 4.2	132.3 ± 3.2	120.1 ± 3.0	<0.0001
Total:HDL-cholesterol ratio	2616	4.05 ± 0.05	4.05 ± 0.06	4.18 ± 0.06	4.13 ± 0.05	4.11 ± 0.05	0.45
Fasting glucose (mg/dL)	2617	95.1 ± 0.9	98.5 ± 0.9	98.2 ± 1.0	99.8 ± 1.0	97.3 ± 1.2	0.39
Fasting insulin (μmol/L)	2614	7.21 ± 0.24	7.41 ± 0.23	6.91 ± 0.22	6.97 ± 0.20	6.55 ± 0.18	0.02
Systolic blood pressure (mm Hg)	2617	127.3 ± 0.8	127.9 ± 1.0	125.9 ± 1.0	125.0 ± 0.8	124.3 ± 0.8	0.01
Diastolic blood pressure (mm Hg)	2617	72.9 ± 0.4	72.8 ± 0.5	71.6 ± 0.5	71.3 ± 0.4	70.8 ± 0.4	0.0003

[1] Mean ± SE values were determined by using linear regression with robust variance estimators and adjusted for age (y), sex, race-ethnicity (white, black, Hispanic, Chinese), education (<high school, high school, some college, college graduate), field center (6 sites), smoking status (never, former, current), prevalent diabetes (yes, no), alcohol use (drinks/wk), physical activity (metabolic equivalent min/wk), BMI (kg/m^2), and waist circumference (cm). 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. MESA, Multi-Ethnic Study of Atherosclerosis.

[2] The numbers of participants with available measures for each biomarker are shown.

[3] Tests for trend (monotonic dose-response) across quintiles were performed by assigning each participant the median fatty acid value in his or her quintile and modeling this variable as a continuous term.

[4] Values are medians; ranges in parentheses.

[5] Mean ± SD (all such values).

TABLE 4
Incidence of type 2 diabetes according to plasma phospholipid *trans*-palmitoleic acid among 2281 US adults in the MESA[1]

	Quintile of *trans*-palmitoleic acid					
	1	2	3	4	5	*P*-trend[2]
trans-Palmitoleic acid (% of fatty acids)[3]	0.03 (0.01–0.03)	0.04 (0.4)	0.05 (0.5)	0.07 (0.06–0.07)	0.10 (0.08–0.24)	
No. of participants[4]	592	317	343	546	495	
Person-years of follow-up	2794	1492	1645	2575	2370	
No. of incident cases	54	26	27	64	34	
Multivariable-adjusted HR (95% CI)						
Model 1[5]	1.0 (reference)	0.76 (0.47, 1.24)	0.66 (0.40, 1.09)	0.89 (0.59, 1.36)	0.52 (0.32, 0.84)	0.02
Model 2[6]	1.0 (reference)	0.77 (0.47, 1.25)	0.66 (0.40, 1.10)	0.89 (0.58, 1.35)	0.52 (0.32, 0.85)	0.02

[1] MESA, Multi-Ethnic Study of Atherosclerosis.
[2] Tests for trend (monotonic dose-response) across quintiles were performed by assigning each participant the median fatty acid value in his or her quintile and modeling this variable as a continuous term.
[3] Values are medians; ranges in parentheses.
[4] On the basis of the distribution of *trans*-palmitoleic acid concentrations, the numbers of participants in each quintile modestly varied.
[5] Adjusted for age (y), sex, race-ethnicity (white, black, Hispanic, Chinese), education (<high school, high school, some college, college graduate), field center (6 sites), smoking status (never, former, current), alcohol use (drinks/wk), physical activity (metabolic equivalent min/wk), BMI (kg/m^2), and waist circumference (cm).
[6] Further adjusted for dietary consumption of whole-fat dairy foods (servings/wk), low-fat dairy foods (servings/wk), red meat (servings/wk), and total energy (kcal/d).

evidence for effect modification by race-ethnicity, sex, BMI, waist circumference, or alcohol use (*P*-interaction >0.20 for each).

In multivariable-adjusted analyses (covariates as in model 2, Table 4), concentrations of 15:0 were associated with a nonsignificant trend toward a lower risk of diabetes (quintile 5—HR: 0.70; 95% CI: 0.42, 1.15; *P*-trend = 0.09). In similarly multivariable-adjusted analyses, 14:0 was not associated with incident diabetes (quintile 5—HR: 0.98; 95% CI: 0.63, 1.53; *P*-trend = 0.80). When 14:0, 15:0, and *trans*-palmitoleate were each mutually adjusted in the same multivariable model, their associations were not substantially altered: HRs (95% CI) in the highest compared with lowest quintile were 1.08 for 14:0 (0.67, 1.74), 0.75 for 15:0 (0.42, 1.32), and 0.58 for *trans*-palmitoleate (0.35, 0.95).

In a meta-analysis combining the present results from the MESA with our prior findings from CHS (5), we found a strong inverse relation between *trans*-palmitoleate concentrations and incidence of diabetes (**Table 5**). Because the quintile concentrations of *trans*-palmitoleate differed between the CHS and the MESA, we also performed dose-response meta-analyses on the basis of *1*) continuous analyses of *trans*-palmitoleate in each cohort and *2*) generalized least squares for trend estimation. Each of these methods accounts for differences in concentrations of *trans*-palmitoleate across cohorts. Evaluated continuously, each 0.05 percentage point of higher *trans*-palmitoleate in the MESA

TABLE 5
Meta-analysis of phospholipid trans-palmitoleic acid and incident diabetes mellitus among 5266 US adults in the MESA and the CHS[1]

	Cohort-specific quintiles of *trans*-palmitoleic acid					
	1	2	3	4	5	*P*-trend[2]
Person-years of follow-up	8389	6927	7201	8386	7839	
No. of incident cases	147	94	86	110	72	
Multivariable-adjusted HR (95% CI)[3]	1.0 (reference)	0.78 (0.58, 1.05)	0.79 (0.58, 1.09)	0.62 (0.46, 0.84)	0.44 (0.31, 0.63)	<0.001

[1] Risk estimates in each quintile were combined by using inverse-variance weighted meta-analysis with fixed effects. The I^2 values for the pooled estimates in quintiles 2–5 were 0%, 0%, 84%, and 0%, respectively. The evidence for heterogeneity in the pooled analysis for quintile 4 (*P*-heterogeneity = 0.01) was consistent with the HR of 0.41 (95% CI: 0.26, 0.64) in the CHS and 0.89 (95% CI: 0.58, 1.35) in the MESA. As shown in the table, pooling of these study-specific findings resulted in a clear monotonic relation between *trans*-palmitoleic acid and incident diabetes. Findings using random-effects models were very similar. CHS, Cardiovascular Health Study; GLST, generalized least-squares models for trend; MESA, Multi-Ethnic Study of Atherosclerosis.
[2] Assessed by using GLST, which uses the multiple data points across quintiles in both cohorts simultaneously to assess dose-response, accounting for the HRs, CIs, and absolute differences in exposure levels across quintiles in each cohort. GLST meta-analysis resulted in a pooled multivariable-adjusted HR of 0.66 (95% CI: 0.56, 0.77) for each 0.05 percentage point of higher *trans*-palmitoleate concentrations.
[3] HRs in the MESA were adjusted for age (y), sex, race-ethnicity (white, black, Hispanic, Chinese), education (<high school, high school, some college, college graduate), field center (6 sites), smoking status (never, former, current), alcohol use (drinks/wk), physical activity (metabolic equivalent min/wk), BMI (kg/m^2), waist circumference (cm), and dietary consumption of whole-fat dairy foods (servings/wk), low-fat dairy foods (servings/wk), red meat (servings/wk), and total energy (kcal/d). HRs in the CHS were adjusted for age (y), sex, race (white, nonwhite), education (<high school, high school, some college, college graduate), field center (4 sites), smoking status (never, former, current), BMI (kg/m^2), waist circumference (cm), coronary heart disease (yes, no), physical activity (kcal/wk), alcohol use (6 categories), and dietary consumption of carbohydrate (% of energy), protein (% of energy), red meat (servings/wk), whole-fat dairy foods (6 categories), low-fat dairy foods (5 categories), and total energy (kcal/d).

was associated with a 32% lower risk of diabetes (multivariable-adjusted HR: 0.68; 95% CI: 0.50, 0.93). In the CHS, each 0.05 higher percentage point was associated with a 28% lower risk (multivariable-adjusted HR: 0.72; 95% CI: 0.61, 0.86). Pooling these results, each 0.05 percentage point of higher *trans*-palmitoleate was associated with a 29% lower incidence of diabetes (pooled HR: 0.71; 95% CI: 0.61, 0.83; $P < 0.001$), with no evidence for heterogeneity between cohorts ($I^2 = 0\%$). By using generalized least squares for trend estimation across quintiles in both cohorts, each 0.05 higher percentage point was associated with a 34% lower risk of incident diabetes (pooled HR: 0.66; 95% CI: 0.56, 0.77; $P < 0.001$).

DISCUSSION

In this large, multiethnic cohort, higher circulating *trans*-palmitoleate concentrations were associated with several metabolic risk factors including higher LDL but also lower triglycerides, lower fasting insulin, and lower blood pressure. In prospective analyses, *trans*-palmitoleate was associated with substantially lower incident diabetes, including a 2-fold lower risk in the highest quintile. All of these relations were similar in men and women and across different race-ethnic subgroups.

These findings are generally consistent with our prior observations in the CHS and the separate Nurses' Health Study (NHS) (5). In the CHS, circulating *trans*-palmitoleate concentrations were associated with lower triglycerides, fasting insulin, and incident diabetes, with an ~2-fold lower risk in the highest quintile. In the NHS, plasma *trans*-palmitoleate was associated with higher HDL, a lower total to HDL-cholesterol ratio, and lower glycated hemoglobin (but not lower triglycerides, although most NHS samples were nonfasting) (5). The present results build on and extend these prior findings by confirming significant inverse associations between *trans*-palmitoleate and triglycerides, fasting insulin, and incident diabetes in another independent cohort. Some differences are evident: for example, *trans*-palmitoleate was associated with higher LDL and lower blood pressure in the MESA but not in the CHS or the NHS. Reasons for these differences are unclear. Yet, the other similarities are notable, given the many differences between participants in these cohorts. The CHS comprised largely older white men and women with a mean age of 75 y at baseline; the NHS comprised highly educated white women aged 60 y at baseline, and the MESA included a broad mix of races and ethnicities, with a mean age of 62 y at baseline.

Whether observed lower metabolic risk relates to *trans*-palmitoleate itself or other components of dairy fat remains unclear. Self-reported dairy intake was not significantly associated with incident diabetes in MESA (11) and was weakly associated in the CHS (5). The stronger findings for *trans*-palmitoleate could relate to its objective measurement as a circulating biomarker, compared with self-reported dairy intake. Yet, 15:0 and 14:0, other biomarkers of dairy consumption, were weakly associated or not associated with incident diabetes, suggesting that the association might be more specific to *trans*-palmitoleate.

In animal models, upregulation of adipose-derived *cis*-palmitoleate, the sister (isomeric) fatty acid of *trans*-palmitoleate, directly improves hepatic and peripheral insulin resistance and suppresses hepatic de novo lipogenesis (6). We previously hypothesized that circulating palmitoleate derived from nonhepatic sources might provide counterregulatory feedback against hepatic fat synthesis and improve peripheral insulin resistance, and that, in this light, *trans*-palmitoleate may mimic a putative counterregulatory role of *cis*-palmitoleate produced outside the liver (5). Recently, gluteofemoral adipose tissue was found to synthesize and release more *cis*-palmitoleate than abdominal adipose tissue, and circulating nonesterified *cis*-palmitoleate (which would be largely derived from adipose tissue) positively correlated with insulin sensitivity (25). These findings support the concept of a potentially protective metabolic role of nonhepatic *cis*-palmitoleate, and our present results are consistent with our hypothesis that *trans*-palmitoleate derived from the diet might share some of these features.

trans-Palmitoleate concentrations in the present analysis (median values: 0.03–0.10 percent of total fatty acids across quintiles) were lower than in the CHS (median values: 0.12–0.26). The consumption of whole-fat dairy, a source of *trans*-palmitoleate, was also lower in the MESA (mean: 5.5 servings/wk) than in the CHS (7.1 servings/wk). Laboratory techniques for fatty acid measures were also not identical in these cohorts (*see* Supplementary Materials under "Supplemental data" in the online issue), which could lead to some relative differences in fatty acid concentrations, especially trace fatty acids such as this one. Yet, even with these differences, as well as substantial other differences between participants in these cohorts, the dose-responses were consistent. For instance, each 0.05% higher *trans*-palmitoleate concentration was associated with a 32% lower risk of diabetes in the MESA and a 28% lower risk in the CHS.

In both the present study and the CHS, *trans*-palmitoleate correlated with reported whole-fat dairy consumption as well as with other circulating biomarkers of dairy fat. In comparison, the correlation between *trans*-palmitoleate and *t*18:1 was stronger in the MESA ($r = 0.70$) than in the CHS ($r = 0.15$). Both of these fatty acids can be consumed from either natural (predominantly dairy) sources or partially hydrogenated oils. In the CHS, fatty acid concentrations were measured ~10 y earlier (1992–1993) than in the MESA (2000–2002). During this time, product reformulations were undertaken in the United States to reduce *trans* fat, so that when fatty acids were measured in the MESA, there may have been relatively less exposure to partially hydrogenated oils. Consistent with this, circulating concentrations of *trans* 18:1 were lower in the MESA (1.3%) than in the CHS (2.0%). With less *trans*18:1 coming from partially hydrogenated oils, a relatively greater proportion would come from natural (ie, dairy) sources. In support of this, consumption of dairy foods correlated with *trans* 18:1 in MESA ($r = 0.24$, $P < 0.001$) but not in the CHS ($P > 0.05$) (23). In combination, these findings suggest a greater proportion of *trans* 18:1 exposure from dairy foods in the MESA compared with the CHS, resulting in a greater degree of common exposure to both *trans*-palmitoleate and *trans* 18:1 due to dairy intake.

Few prior studies have evaluated *trans*-palmitoleate or other dairy fatty acid biomarkers and incident diabetes. In a nested case-control study in Sweden, higher erythrocyte 15:0 and 17:0, but not 14:0, was associated with lower diabetes risk (26). Yet, results were minimally adjusted for potential confounding factors, and *trans*-palmitoleate was not assessed. In a German cohort with more extensive multivariate adjustment, higher erythrocyte 14:0, 15:0, 17:0, and *trans*-palmitoleate each tended toward lower diabetes risk (highest-quintile HRs: 0.74–0.89), but none were

statistically significant (P-trend > 0.10 each) (27). In an English cohort, phospholipid and erythrocyte 17:0, but not 14:0 or 15:0, was associated with lower diabetes risk; phospholipid 14:0, which can also be formed from de novo lipogenesis, was linked to a higher risk (28). This pilot study contained relatively few cases (n = 199), and *trans*-palmitoleate (*trans* 16:1n–7) was not assessed. If future intervention studies confirm that *trans*-palmitoleate reduces diabetes, then various potential strategies could be envisioned, such as enrichment of milk and other dairy products with this fatty acid, or development and testing of supplement sources. The present findings suggest that this fatty acid is a robust observational biomarker of diabetes risk and support the need for further investigation of the potential causality of this association.

Our analysis has several strengths. Information on fatty acids, metabolic risk factors, covariates, and diabetes incidence was prospectively collected in a well-established multicenter study with close follow-up. Biomarker measures provided an objective assessment of exposure. The diverse racial-ethnic mix of the cohort and similar findings by sex and across race-ethnic groups increase confidence in the validity and generalizability of the findings. We adjusted for a number of demographic, lifestyle, and dietary covariates, minimizing residual confounding.

Potential limitations should be considered. Associations with metabolic risk factors were cross-sectional, which limited the assessment of temporality. However, prospective analyses of diabetes incidence were consistent with the cross-sectional findings. Measurement error and biological variability were present in covariates, which could bias results in unpredictable directions, including causing residual confounding. However, the observed magnitude of the findings, including a 2-fold lower risk of diabetes, makes it unlikely that residual confounding fully accounts for these relations. In addition, the observed associations were independent of estimated dairy food consumption and of other dairy fatty acid biomarkers. *trans*-Palmitoleate was measured with laboratory and biological (ie, temporal) imprecision. Laboratory imprecision was largest at low concentrations (eg, quintiles 1 and 2), but this would not appreciably affect discrimination of participants across extreme (lowest compared with highest) quintiles. Also, in prospective analyses, laboratory misclassification would improbably relate to future incidence of diabetes. Thus, these errors would attenuate findings, leading to underestimation of true relations. Such effects would be largest in the midquintiles (2–4), so observed relations in quintile 5 compared with quintile 1 might be most reliable. *trans*-Palmitoleate concentrations were not normally distributed, but findings were consistent in direction and magnitude in categorical and continuous analyses.

In summary, our findings suggest that higher circulating *trans*-palmitoleate is a biomarker of lower diabetes risk in a multiethnic cohort of US adults. These results support the need for detailed experimental and clinical investigations to determine whether this observed relation is causal and if altering the circulating concentrations of this fatty acid—for example, by means of dietary interventions—will reduce the risk of diabetes.

We thank the participants of the MESA study, the Coordinating Center, MESA investigators, and study staff for their valuable contributions. A full list of participating MESA investigators and institutions can be found at http://www.mesa-nhlbi.org.

The authors' responsibilities were as follows—DM: conception and design, obtainment of funding, data collection, statistical analysis, data interpretation, manuscript drafting, manuscript critical revision, and approval of the final submitted manuscript; MCdOO: statistical analysis, data interpretation, critical revision of the manuscript, and approval of the final submitted manuscript; RNL, AMF, and DSS: data interpretation, critical revision of the manuscript, and approval of the final submitted manuscript; MYT: obtainment of funding, data collection, data interpretation, critical revision of the manuscript, and approval of the final submitted manuscript; GH: critical revision of the manuscript and approval of the final submitted manuscript; JAN: conception and design, obtainment of funding, statistical analysis, data interpretation, manuscript drafting, critical revision of the manuscript, and approval of the final submitted manuscript. DM and GH reported a provisional patent application, filed by Harvard University and assigned to Harvard University, listing them as coinventors to the US Patent and Trademark Office for use of *trans*-palmitoleic acid to prevent and treat insulin resistance, type 2 diabetes, and related conditions. DM reported ad hoc travel reimbursement or honoraria for speaking on diet and cardiometabolic risk from the International Life Sciences Institute, Bunge, Quaker Oats, Pollock Institute, Life Sciences Research Organization, and Nutrition Impact; scientific advisory board membership for Unilever North America; and ad hoc consulting fees for discussing the science on diet and cardiometabolic risk from Foodminds. None of the other authors reported a conflict of interest.

REFERENCES

1. Tong X, Dong JY, Wu ZW, Li W, Qin LQ. Dairy consumption and risk of type 2 diabetes mellitus: a meta-analysis of cohort studies. Eur J Clin Nutr 2011.
2. Choi HK, Willett WC, Stampfer MJ, Rimm E, Hu FB. Dairy consumption and risk of type 2 diabetes mellitus in men: a prospective study. Arch Intern Med 2005;165:997–1003.
3. Liu S, Choi HK, Ford E, Song Y, Klevak A, Buring JE, Manson JE. A prospective study of dairy intake and the risk of type 2 diabetes in women. Diabetes Care 2006;29:1579–84.
4. van Dam RM, Hu FB, Rosenberg L, Krishnan S, Palmer JR. Dietary calcium and magnesium, major food sources, and risk of type 2 diabetes in U.S. black women. Diabetes Care 2006;29:2238–43.
5. Mozaffarian D, Cao H, King IB, Lemaitre RN, Song X, Siscovick DS, Hotamisligil GS. Trans-palmitoleic acid, metabolic risk factors, and new-onset diabetes in U.S. adults: a cohort study. Ann Intern Med 2010;153:790–9.
6. Cao H, Gerhold K, Mayers JR, Wiest MM, Watkins SM, Hotamisligil GS. Identification of a lipokine, a lipid hormone linking adipose tissue to systemic metabolism. Cell 2008;134:933–44.
7. Dimopoulos N, Watson M, Sakamoto K, Hundal HS. Differential effects of palmitate and palmitoleate on insulin action and glucose utilization in rat L6 skeletal muscle cells. Biochem J 2006;399:473–81.
8. Sauma L, Stenkula KG, Kjolhede P, Stralfors P, Soderstrom M, Nystrom FH. PPAR-gamma response element activity in intact primary human adipocytes: effects of fatty acids. Nutrition 2006;22:60–8.
9. Maedler K, Oberholzer J, Bucher P, Spinas GA, Donath MY. Monounsaturated fatty acids prevent the deleterious effects of palmitate and high glucose on human pancreatic beta-cell turnover and function. Diabetes 2003;52:726–33.
10. Erbay E, Babaev VR, Mayers JR, Makowski L, Charles KN, Snitow ME, Fazio S, Wiest MM, Watkins SM, Linton MF, et al. Reducing endoplasmic reticulum stress through a macrophage lipid chaperone alleviates atherosclerosis. Nat Med 2009;15:1383–91.
11. Nettleton JA, Steffen LM, Ni H, Liu K, Jacobs DR Jr. Dietary patterns and risk of incident type 2 diabetes in the Multi-Ethnic Study of Atherosclerosis (MESA). Diabetes Care 2008;31:1777–82.
12. Bild DE, Bluemke DA, Burke GL, Detrano R, Diez Roux AV, Folsom AR, Greenland P, Jacob DR Jr, Kronmal R, Liu K, et al. Multi-ethnic study of atherosclerosis: objectives and design. Am J Epidemiol 2002;156:871–81.
13. Nettleton JA, Steffen LM, Mayer-Davis EJ, Jenny NS, Jiang R, Herrington DM, Jacobs DR Jr. Dietary patterns are associated with biochemical markers of inflammation and endothelial activation in the Multi-Ethnic Study of Atherosclerosis (MESA). Am J Clin Nutr 2006;83:1369–79.
14. Cushman M, Cornell ES, Howard PR, Bovill EG, Tracy RP. Laboratory methods and quality assurance in the Cardiovascular Health Study. Clin Chem 1995;41:264–70.

15. Steffen BT, Steffen LM, Tracy R, Siscovick D, Jacobs D, Liu K, He K, Hanson NQ, Nettleton JA, Tsai MY. Ethnicity, plasma phospholipid fatty acid composition and inflammatory/endothelial activation biomarkers in the Multi-Ethnic Study of Atherosclerosis (MESA). Eur J Clin Nutr 2012;66:600–5.
16. Wolk A, Vessby B, Ljung H, Barrefors P. Evaluation of a biological marker of dairy fat intake. Am J Clin Nutr 1998;68:291–5.
17. Wolk A, Furuheim M, Vessby B. Fatty acid composition of adipose tissue and serum lipids are valid biological markers of dairy fat intake in men. J Nutr 2001;131:828–33.
18. Brevik A, Veierod MB, Drevon CA, Andersen LF. Evaluation of the odd fatty acids 15:0 and 17:0 in serum and adipose tissue as markers of intake of milk and dairy fat. Eur J Clin Nutr 2005;59:1417–22.
19. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499–502.
20. Mayer-Davis EJ, Vitolins MZ, Carmichael SL, Hemphill S, Tsaroucha G, Rushing J, Levin S. Validity and reproducibility of a food frequency interview in a multi-cultural epidemiology study. Ann Epidemiol 1999;9:314–24.
21. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials 1986;7:177–88.
22. Greenland S, Longnecker MP. Methods for trend estimation from summarized dose-response data, with applications to meta-analysis. Am J Epidemiol 1992;135:1301–9.
23. Micha R, King IB, Lemaitre RN, Rimm EB, Sacks F, Song X, Siscovick DS, Mozaffarian D. Food sources of individual plasma phospholipid trans fatty acid isomers: the Cardiovascular Health Study. Am J Clin Nutr 2010;91:883–93.
24. Warnet JM, Cambien F, Vernier V, Pecoraro M, Flament C, Ducimetiere P, Jacqueson A, Richard JL, Claude JR. Relation between consumption of alcohol and fatty acids esterifying serum cholesterol in healthy men. Br Med J (Clin Res Ed) 1985;290:1859–61.
25. Pinnick KE, Neville MJ, Fielding BA, Frayn KN, Karpe F, Hodson L. Gluteofemoral adipose tissue plays a major role in production of the lipokine palmitoleate in humans. Diabetes 2012;61:1399–403.
26. Krachler B, Norberg M, Eriksson JW, Hallmans G, Johansson I, Vessby B, Weinehall L, Lindahl B. Fatty acid profile of the erythrocyte membrane preceding development of type 2 diabetes mellitus: nutrition, metabolism, and cardiovascular diseases. Nutr Metab Cardiovasc Dis 2008;18:503–10.
27. Kröger J, Zietemann V, Enzenbach C, Weikert C, Jansen EH, Doring F, Joost HG, Boeing H, Schulze MB. Erythrocyte membrane phospholipid fatty acids, desaturase activity, and dietary fatty acids in relation to risk of type 2 diabetes in the European Prospective Investigation into Cancer and Nutrition (EPIC)–Potsdam Study. Am J Clin Nutr 2011;93:127–42.
28. Patel PS, Sharp SJ, Jansen E, Luben RN, Khaw KT, Wareham NJ, Forouhi NG. Fatty acids measured in plasma and erythrocyte-membrane phospholipids and derived by food-frequency questionnaire and the risk of new-onset type 2 diabetes: a pilot study in the European Prospective Investigation into Cancer and Nutrition (EPIC)–Norfolk cohort. Am J Clin Nutr 2010;92:1214–22.